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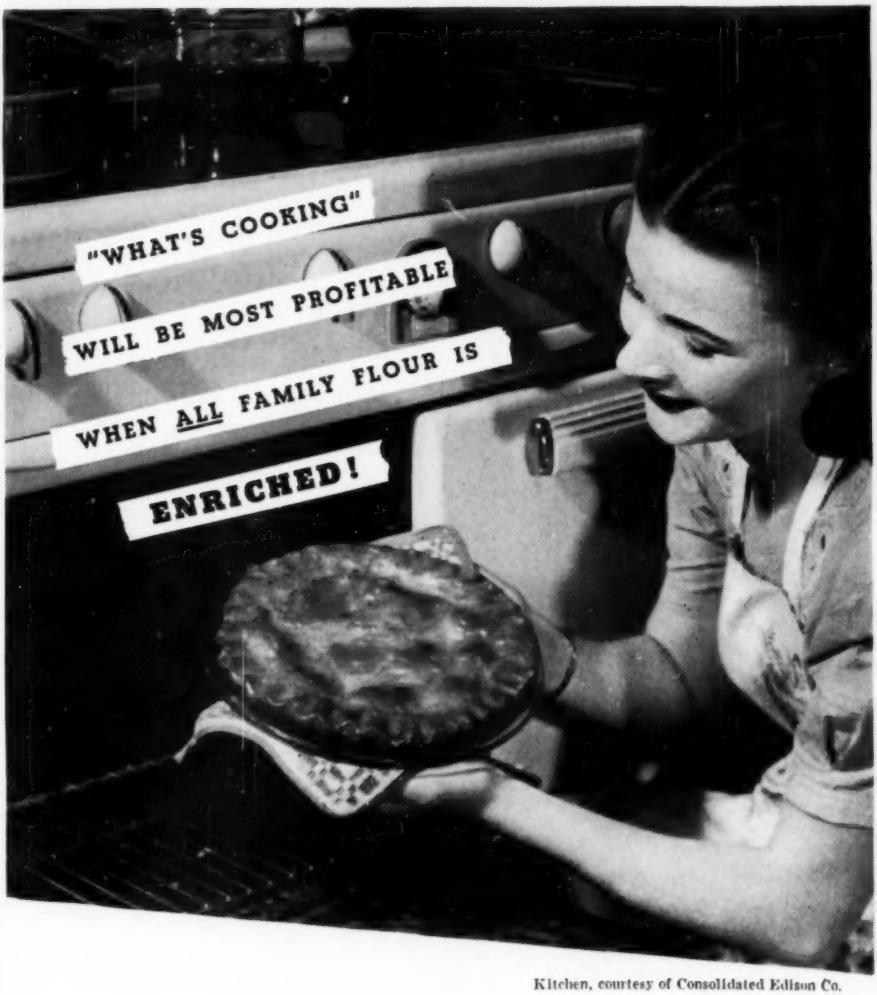

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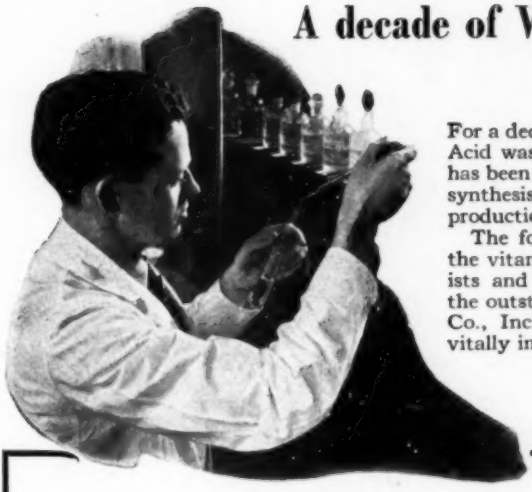

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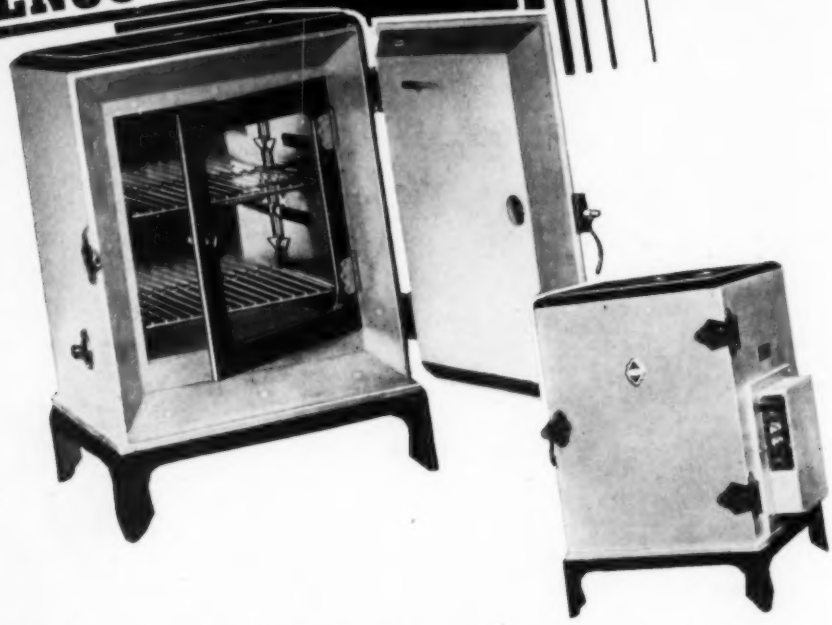

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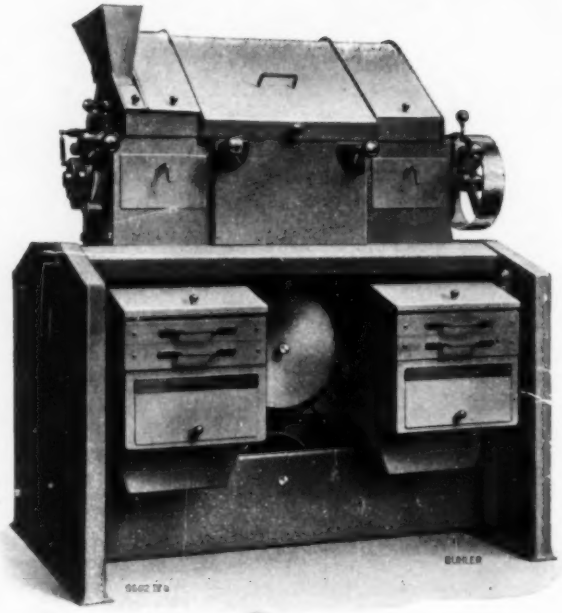

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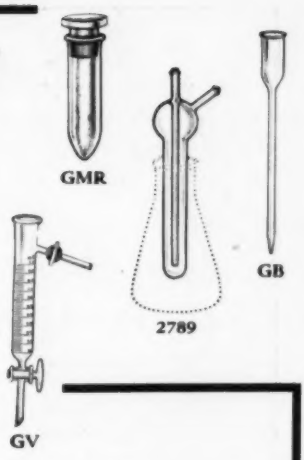

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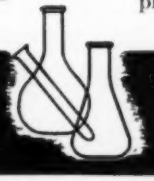

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CEREAL CHEMISTRY

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EFFECT OF THE SULFUROUS ACID STEEP IN CORN WET MILLING

MARY J. COX, M. M. MACMASTERS, and G. E. HILBERT

Starch and Dextrose Division, Northern Regional Research Laboratory,[1] Peoria, Illinois

(Presented at the Annual Meeting, May 1944; received for publication, June 23, 1944)

In the wet-milling process for the manufacture of corn starch it is customary to steep whole corn kernels, prior to milling, in dilute sulfurous acid solution for from 38 to 60 hr, depending upon the moisture, age, and type of corn. Too much emphasis cannot be placed upon the importance of steeping for ensuring smooth milling operation and maximum yield of prime-quality starch. According to Bartling (1940) the chief purposes of the steep are: (1) to soften the corn kernel, (2) to reduce or inhibit the activity of microorganisms, and (3) to whiten the starch. The softening action is apparently of the greatest importance. Regarding the mechanism of the action of aqueous sulfur dioxide little or nothing is known, although it has been widely assumed in the industry that dispersion of the protein is facilitated (*cf.* Brössler, 1895) with consequent loosening of the starch granules from the surrounding proteinaceous material. As far as is known, however, no studies have been reported on the function of sulfur dioxide in the steep.

Information on the specific action of the sulfurous acid in the steep would provide a logical basis in the search for superior steeping agents or for developing new processing methods. Experiments were therefore undertaken having three main objectives: (1) to determine the degree to which sulfurous acid in the steep aids in the softening of corn kernels, (2) to ascertain the starch-protein relations within the corn kernel, and the effect of the sulfurous acid steep upon them, and (3) to determine what, if any, effect the concentration of sulfurous acid in the steep has upon the starch subsequently extracted from the corn.

Since direct physical or chemical methods for following softening action are lacking, the simplest and most satisfactory approach ap-

[1] This is one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

peared to be by microscopic examination of changes produced under various steeping conditions. This method was used and the results correlated with those of starch extraction studies.

Experimental and Discussion

Corn Endosperm. Endosperm cells vary greatly in size. Just under the aleurone layer the storage cells in the dent corn (Iowa 939) studied were found to measure approximately 50 × 35 μ (radial diameter × tangential diameter, respectively). (*Cf.* Fig. 1.) Only about 2 to 8 rows of cells were this small; centripetal to these lay others, very irregular in size, ranging from approximately 80 × 50 μ to 240 × 100 μ (Fig. 2). In some kernels no further differentiation was observed, but in others there was a row of flattened cells at the center of the kernel, approximately 55 × 70 μ to 50 × 150 μ. In sectioning, some cells are cut through the center while others are cut more or less toward one end; hence cells having the same over-all dimensions may appear to differ considerably in size when the cross-sectional diameters are compared, as in Figures 4, 6, 7, and 8. Cell size also varies somewhat from kernel to kernel. Measurements of cell-wall thickness were made on cross sections of dent corn kernels mounted in distilled water. The average in the horny endosperm was 2 to 5 μ. Cells just under the aleurone had the thickest walls. In commercial starch processing, this cell-wall material contributes heavily to the "fiber" fraction, which is removed by the silk screens prior to the collection of prime-quality starch.

Endosperm cells showed 30 to 85 starch granules exposed by cross sectioning, the number of granules varying with cross-sectional diameters of cells. Small granules in a heavy protein matrix occurred just under the aleurone layer. Nearer the center of the kernel the granules were much larger in size and the protein network less massive. The difference in size of the starch granules is strikingly shown by comparison of Figures 1 and 2. The granules immediately under the aleurone layer are of the smallest size. Within a more or less narrow region, the granules are larger in diameter the greater their distance from the aleurone layer. Granules 3 to 9 cells distant from the aleurone layer are about equal in size to those in cells near the center of the kernel. Most of the starch granules lying close to the aleurone layer are contributed during processing to the so-called "tailing" or "bastard" starch, and relatively few to the prime-quality fraction.

Relationships between dent corn endosperm and embryo are shown in Figure 3. For details of kernel structure, see Hayward (1938).

Penetration of the Kernel by the Steep. Several steeps were made in water and in sulfurous acid from which samples of dent corn were with-

drawn at short intervals and examined macroscopically after sectioning to determine the site and degree of penetration of the steep liquor into the kernel. The liquid entered at the chalazal or basal end of the kernel

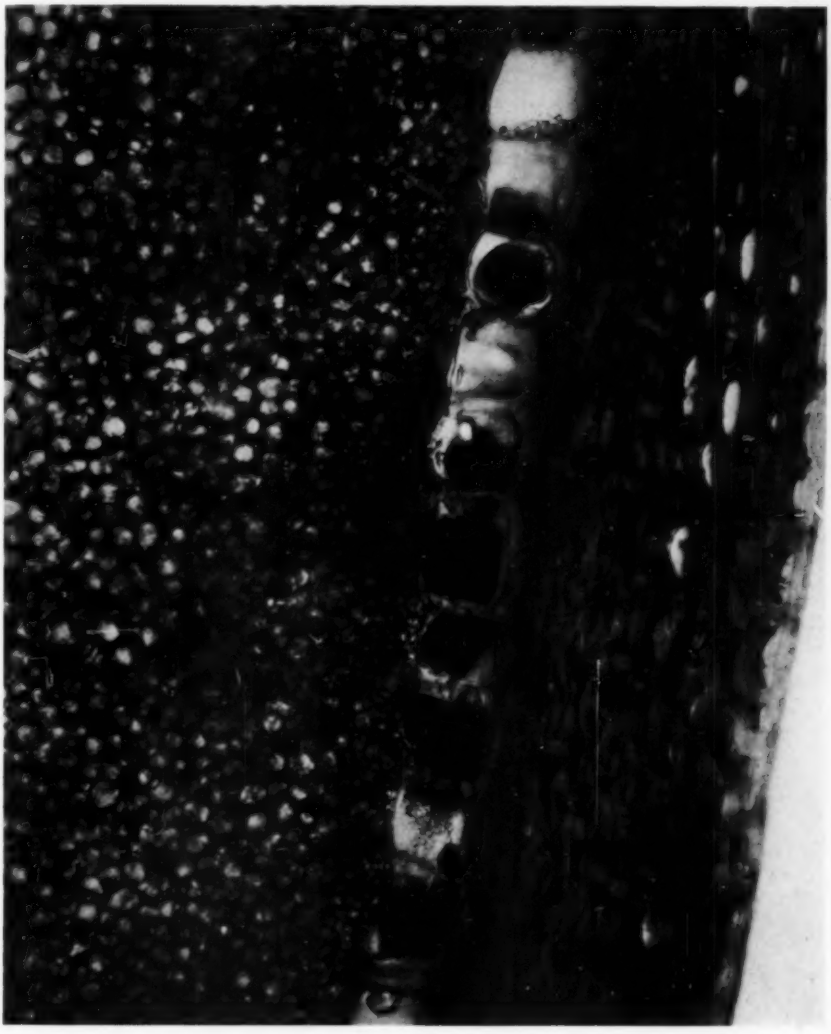

Fig. 1. Cross section approximately midway between base and crown of corn kernel, showing pericarp, aleurone cells (from some of which the contents have been lost in sectioning), and outer endosperm storage cells full of starch granules. 500 ×.

and traveled rapidly upward just under the seed coat. It apparently thus reached the top of the kernel before starting to penetrate through the aleurone layer to the starch cells of the endosperm at the sides to

any appreciable extent. The embryo including the scutellum was penetrated before the endosperm. Somewhat less than 4 hr was required at 49°C for complete wetting of the germ and about 8 hr for complete penetration of the endosperm by the steep. At the end of

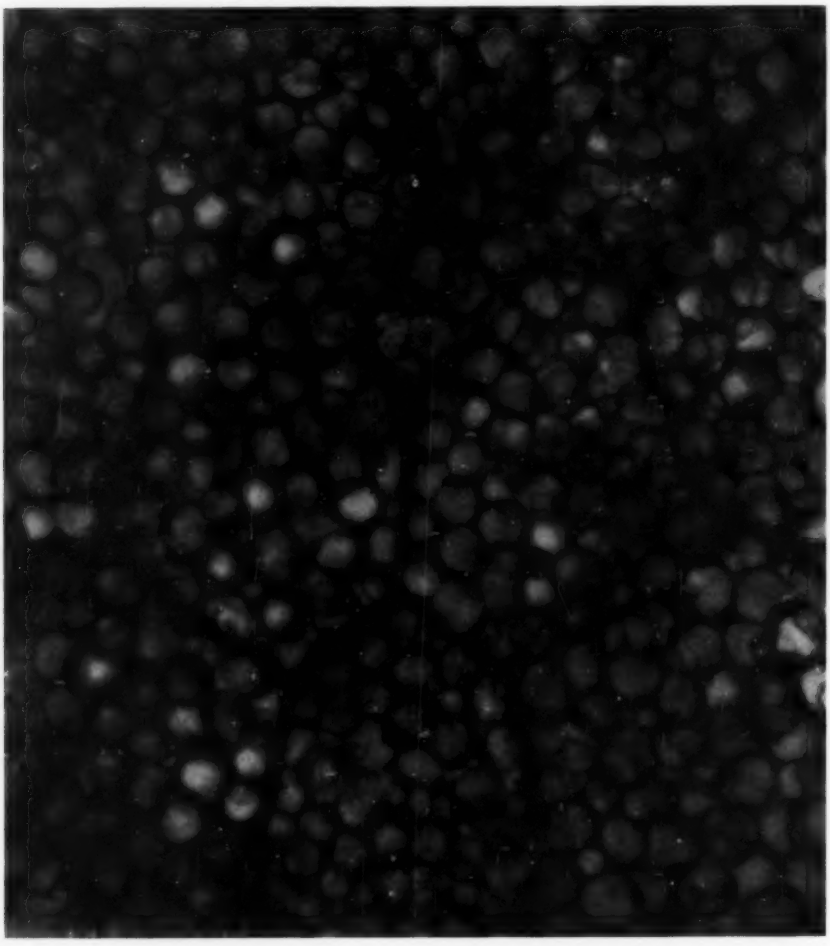

Fig. 2. Cross section near inner edge of horny endosperm, approximately midway between base and crown, showing endosperm storage cells full of starch. 500 ×.

8 hr, however, the kernel was not greatly softened. Corn became progressively easier to grind as the duration of the sulfurous acid steep was increased from 4 to 24 hr.

Starch-Protein Relations. Sections of corn kernels were obtained for microscopic studies by use of a sliding microtome with freezing

attachment. When these sections were stained directly with iodine solution none of the details of the starch-protein relations could be observed owing to the opaqueness of the deeply stained starch granules. The following method, therefore, was devised for removing the starch granules from a section of corn kernel and at the same time staining the protein network and the cell walls: The section was placed in a drop of distilled water on a microscope slide, flooded with 0.2*N* iodine solution, and allowed to stand ½ min; the iodine was pipetted off;

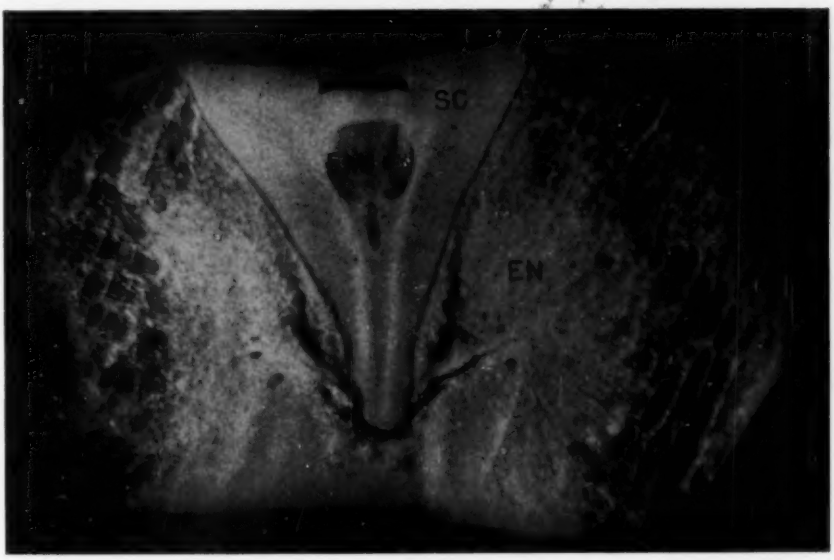

Fig. 3. Cross section through the middle of corn kernel from which the pericarp, commonly known as the seed coat or hull, has been removed. Positions of the embryo (em), including the scutellum (sc), and of the endosperm (en) are indicated. The floury endosperm near the scutellum has been torn slightly in sectioning. The cells show clearly in the horny endosperm, but are less distinct in the floury. Approximately 13 ×.

and a drop or two of 70% sulfuric acid was added. After about 1 sec the sulfuric acid was rinsed off with distilled water and the section washed several times to remove the starch. The sulfuric acid gelatinized the starch granules and, in consequence, they were easily removed from the surrounding protein which remained very tough and hard to break. A drop or two of 0.2*N* iodine solution was then placed on the moist section and the cover slip added. The initial treatment with iodine and sulfuric acid left the cell walls unstained or stained blue depending upon the amount of cellulose present, while the final addition of iodine gave a bright yellow color, characteristic of protein, to the network. At the same time, any starch not removed by the previous washes was colored deep blue-black.

Comparisons were made of sections stained in this manner with others prepared: (1) by mounting directly in cold water, (2) by heating in water to gelatinize the starch granules which thereupon were forced out of the protein network, (3) by removing all loose starch granules by washing with water and subsequently staining the protein network

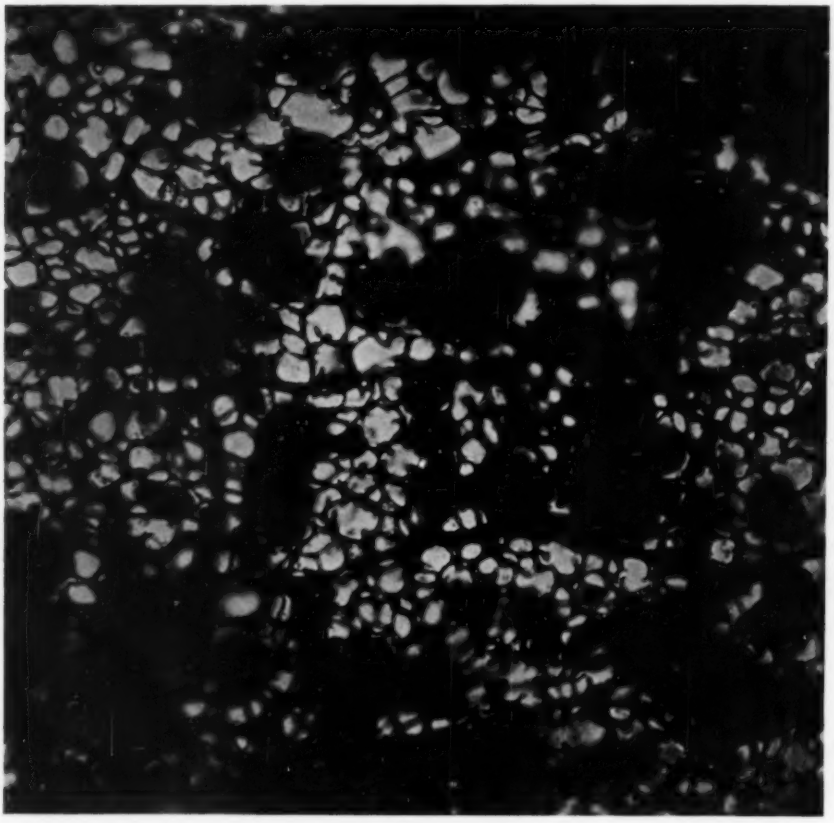

Fig. 4. Cross section of unsteeped dent corn, comparable to that shown in Figure 2 as to position, but with the starch granules gelatinized and largely removed. The proteinaceous network holds them tightly. Those remaining in the network appear as large black bodies having been stained with iodine. The thick dark lines are cell walls; the thinner lines in honeycomb arrangement are the protein network. 500 ×.

with picric acid, and (4) by the use of several combinations of stains commonly employed on plant material. There was no evidence that the microscopic details and relationships of protein, starch, and cell walls were in any way changed by the treatment adopted as standard, other than that the starch was gelatinized and consequently often forced out of the protein network.

Careful microscopic study of cross sections of Iowa Hybrid 939 dent corn endosperm showed that the individual starch granules in both the horny and the floury endosperm lay completely embedded in a matrix composed largely, if not wholly, of protein. In the floury endosperm at the center of dent corn kernels the protein is less dense and each protein envelope fits about the enclosed starch granule much more loosely than in the horny endosperm. When the starch granules were

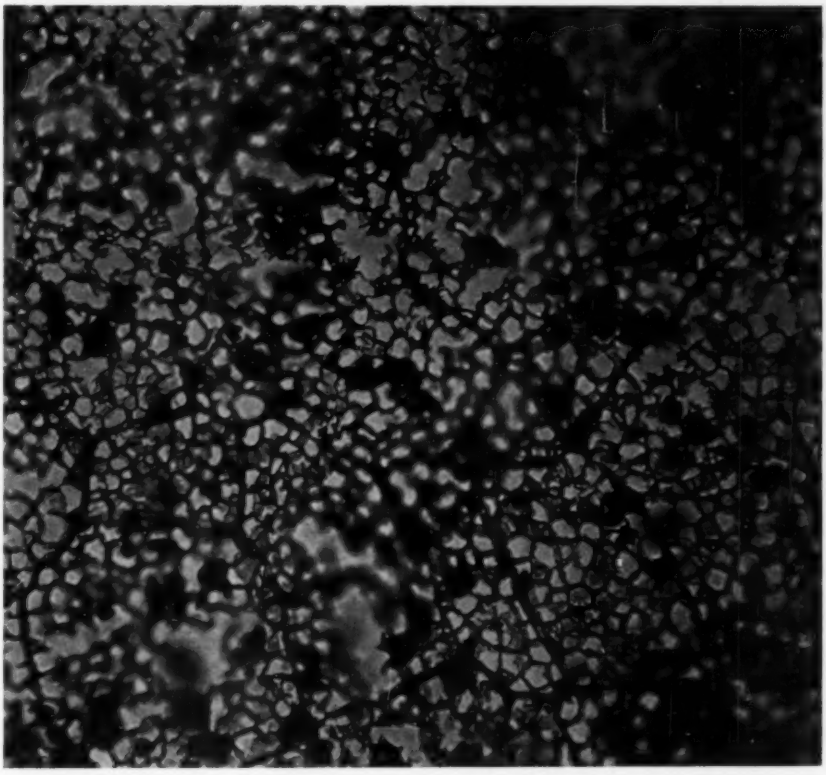

Fig. 5. Cross section of unsteeped popcorn, comparable to that of dent corn shown in Figure 4. The protein network is stronger than in dent corn and has been more completely freed of starch granules without causing damage to the network. Some starch granules, however, still remain. 200 ×.

removed from thin sections (as described above), the empty protein network was clearly visible within the individual cells (Figs. 4 and 5).

In Cuzco, the flour corn studied, there is a heavy protein network just inside the aleurone. This is, however, less dense than in the corresponding area of dent corn. In Cuzco corn the protein matrix becomes progressively weaker from the aleurone to the center where it is found largely in discrete flat particles instead of as a network.

The strands of protein composing the network were found to be birefringent between crossed nicol prisms, indicating that the majority of the molecules in the network, that is, in the protein film, are oriented. In crystalline or oriented high polymeric polar substances it is generally recognized that powerful intermolecular binding forces are operative. As a result, such high polymers are usually strong and difficult to disperse in solvents. The oriented and therefore possibly crystalline nature of the protein network probably accounts for its toughness and for the difficulty encountered in dispersing it. At a magnification of 1200 × the protein appeared homogeneous, without structural detail.

From the microscopic studies it appeared that the protein content was considerably greater in horny than in floury endosperm. This was indirectly confirmed by analytical data obtained previously, and for another purpose, on hand-separated horny and floury endosperm fractions of another typical dent corn, Reid Yellow Dent. These data are summarized in Table I. The 4 to 5% unaccounted for by analysis

TABLE I

COMPOSITION OF HORNY AND FLOURY ENDOSPERM OF REID YELLOW DENT CORN

Constituent	Horny endosperm	Floury endosperm
	%	%
Ash	0.27	0.35
Protein (N × 6.25)	13.25	7.69
Alcohol soluble N	1.49	0.64
Sugar	0.47	0.52
Starch (by polarimetric method)	80.4	85.6
Oil (petroleum ether extract)	0.73	0.33
Undetermined	3.4	4.9

is undoubtedly attributable to cell-wall material. From these data it is apparent that there is almost twice as much protein in horny as in floury endosperm. The ratio of starch to protein is approximately 11 : 1 in floury and 6 : 1 in horny endosperm. Although the microscopic and analytical studies were made upon different varieties of corn, dent corns, in general, appear to be sufficiently similar to warrant correlation of these data.

Correlation of Microscopic and Extraction Studies. Three corns were chosen for the microscopic investigations on steeping. *Iowa Hybrid 939*, a dent variety very popular in the corn belt, grown in Iowa, was used as typical of the type of corn reaching wet millers; it contains both horny and floury endosperm. *White Cuzco* flour corn, grown in Peru, and *South American* variety popcorn, grown in Iowa, were used in a study of possible differences in the effects of steeping on floury and horny endosperm, respectively.

For more detailed studies of the effect of steep upon starch characteristics, *Iowa Hybrid 939* and *Iowax 1*, comparable hybrid corn into which the waxy or glutinous starch character has been bred, were used. The latter was chosen because of wide current interest in glutinous starch for tapioca replacement.

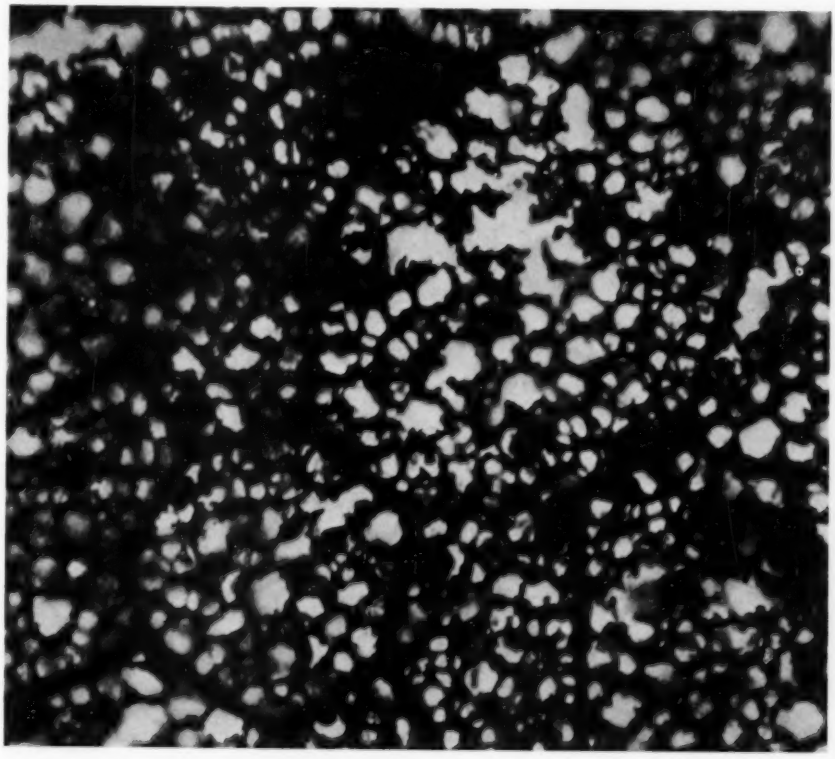

Fig. 6. Cross section of corn kernel comparable to those shown in Figures 4 and 5. Dent corn after 24-hr steep in distilled water. The protein network is somewhat swollen; in parts of the network incipient globule formation is apparent. 500 ×.

Starch was extracted on a laboratory scale by a method analogous to that used commercially for the production of corn starch. Although practice varies considerably from plant to plant, most, if not all, of the wet millers in this country use steeps having sulfur dioxide concentrations of between 0.10 and 0.30%. In order to cover this range and to study more extreme conditions as well, the present experiments were carried out with distilled water and 0.10, 0.20, 0.30, and 0.40% sulfur dioxide steeps. A 24-hr steeping period at 49°C was adopted as standard. In certain instances, as noted below, other steeps were used for special studies. Steeping was carried out by placing 400 g of corn and

1700 ml of steep liquid in a 2-l Erlenmeyer flask in a thermostatically controlled bath. The bottom of the flask had been drawn downward into a shallow inverted cone. Steep liquid was withdrawn from the bottom at the center of the flask (*i.e.*, the lowest point) and returned through a circulating pump to the top of the flask. The steeping system was entirely closed, except for a small vent through the flask stopper. The bath temperature was recorded automatically throughout the steeping period. In sulfurous acid steeps, the sulfur dioxide

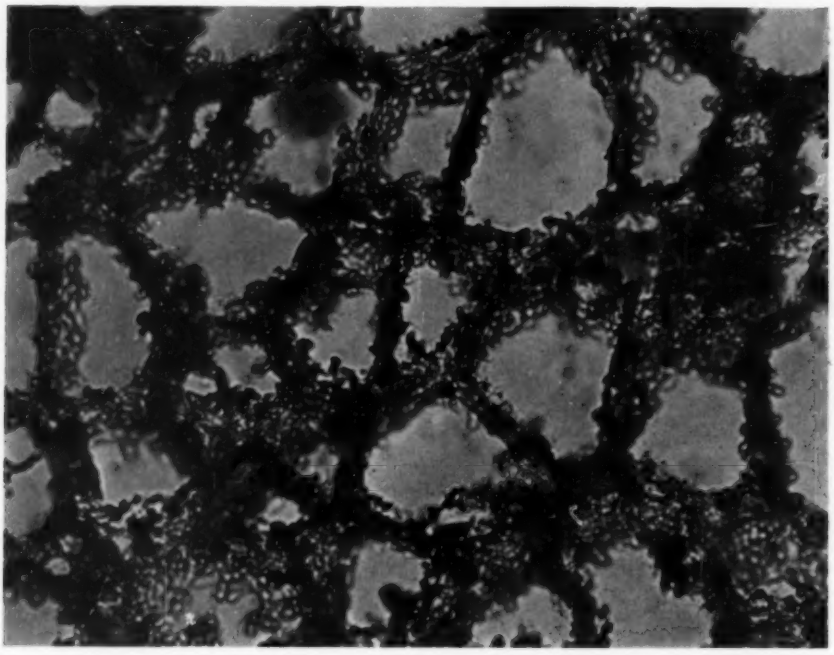

Fig. 7. Cross section of dent corn kernel, comparable to those shown in Figures 4 and 6, after a 24-hr 0.2% SO_2 steep. The cells appear smaller than those in the other figures only because of variations between individual corn kernels. Disintegration of the protein matrix has proceeded so far that the network breaks considerably when the starch granules are removed; the weakened network has collapsed onto the cell walls. 500 ×.

content decreased to about one half its original value during a 24-hr period. Since the effect of only one steep constituent was under investigation, no attempt was made to duplicate a commercial steep, which employs gluten overflow water containing appreciable quantities of substances ("solubles") dissolved from the corn.

After decantation of the steep liquor, the corn was ground through a Hobart sausage mill, screened through No. 17 silk bolting cloth, and the starch collected by centrifuging. This crude starch was separated into prime-quality and "tailings" fractions by resuspending in distilled

water, centrifuging, and scraping the "tailings" fraction layer from the surface of the starch. Washing and separation were repeated 4 to 5 times, or until no layering was obtained on centrifuging. The prime-quality starch was dried in a forced draft oven at 40°C.

Analytical methods and details of the procedure for viscosity determinations have been described elsewhere (MacMasters and Hilbert,

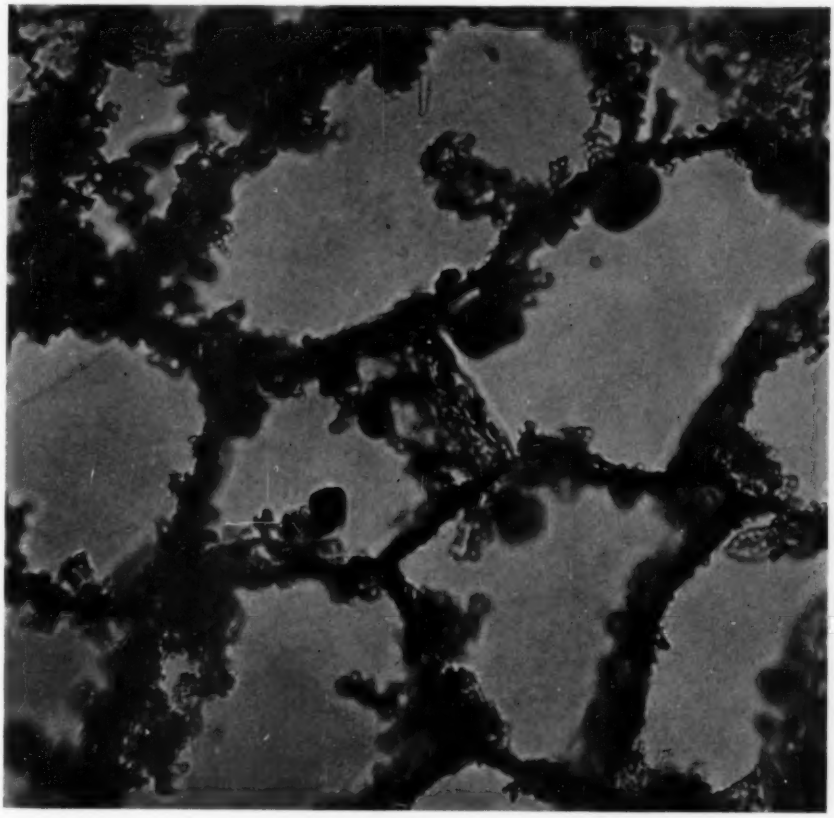

Fig. 8. Portion of cross section of dent corn kernel after a 24-hr 0.4% SO_2 steep. The protein matrix is almost completely disintegrated; the remaining pieces have dropped out during preparation of the section and only those supported by the cell walls are evident. 500 ×.

1944). Viscosities were taken with a Stormer viscometer, and hence are only qualitatively comparable with commercial viscosity data.

After dent corn had been steeped in sulfur dioxide solution, the protein matrix was found to be more or less altered, the degree of change depending upon the concentration of sulfur dioxide in the steep. When distilled water or 0.10% sulfur dioxide steep was used, the protein network was still almost as distinct as originally and apparently almost as

strong. (*Cf.* Figs. 4 and 6.) The network was more swollen, however, and its individual portions often appeared to be made up of tiny globules of hydrated protein. After a 0.20% sulfur dioxide steep, the network was appreciably weakened and partially dispersed. Upon removal of the starch granules, the undispersed protein usually collapsed against the cell walls and appeared as a mass of material with little evidence of the original network films. This is shown in Figure 7. After a 0.4% sulfur dioxide steep, much of the protein had been dispersed, and only a relatively small amount lay along the cell walls after the starch had been removed (Fig. 8). These data are summarized in Table II. They refer to the greater part of the endosperm;

TABLE II

EFFECT OF NATURE OF STEEP ON STARCH-PROTEIN MATRIX RELATIONS AND ON RECOVERY OF STARCH

Steep		Condition of protein in matrix	Starch recovery
Type	Duration		
	hr		%
None	—	Coarse network, holding starch tightly	—
Distilled water	4	Slightly less coarse, but holding starch tightly	—
Distilled water	24	Slightly less coarse than at 4 hr, but holding starch tightly	64
0.1% SO_2	4	Slightly less coarse than unsteeped, holding starch tightly	—
0.1% SO_2	24	Somewhat less coarse than at 4 hr, holding starch tightly	82
0.2% SO_2	8	More dispersed than after 24-hr steep in 0.1% SO_2	—
0.2% SO_2	24	Not appreciably different from 8-hr sample	83
0.3% SO_2	4	Much dispersed	—
0.3% SO_2	24	In separate particles; no network remaining	88
0.4% SO_2	4	Indistinguishable from 4-hr steep in 0.3% SO_2	—
0.4% SO_2	24	Indistinguishable from 4-hr sample	89

just under the aleurone layer, however, the protein matrix appeared more dense and did not disperse so readily. In this region, only hydration, comparable to that shown in Figure 6, was observed after all steeps except the 0.4% sulfur dioxide. After this steep many globules could be seen in the network.

Disintegration of the protein matrix was more rapid in Cuzco corn than in dent (*cf.* Figs. 8, 9, and 10), and slower in popcorn. This would be expected from the comparative amounts of protein present.

Several 0.2% sulfur dioxide steeps were run at temperatures below (38°, 43°) and above (52°, 55°) 49°C to determine what effects temperature of the steep might have. Variations in starch recovery were not

significant under laboratory processing conditions although processing was easier the higher the temperature used. It is well known by the industry, however, that the temperature of the steep employed at the factory has a profound effect upon both the ease of processing and the amount of starch recovered.

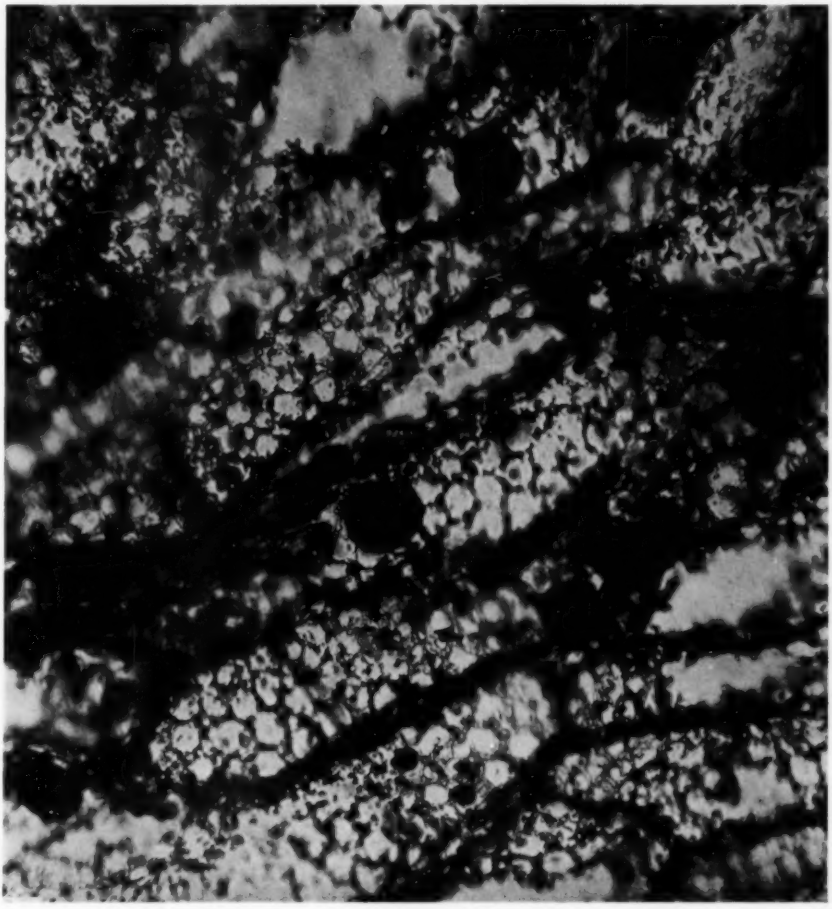

Fig. 9. Portion of cross section of Cuzco corn kernel after a 6½-hr 0.4% SO_2 steep. The protein network is still strong; globule formation is evident. 200 ×.

Microscopic examination showed the protein to be largely in globules after the corn had been steeped at 38° or 43°C. Just under the aleurone layer, only hydration of the protein, with consequent swelling, was evident. After steeping at 52° or 55°C, the protein was largely dispersed; in the cells just under the aleurone layer many protein globules were observed.

When the sulfur dioxide concentration was increased to 0.4%, steeping at 52°C dispersed the protein in most of the endosperm almost completely; in the cells just under the aleurone layer considerable dispersion had occurred, but many globules were still present.

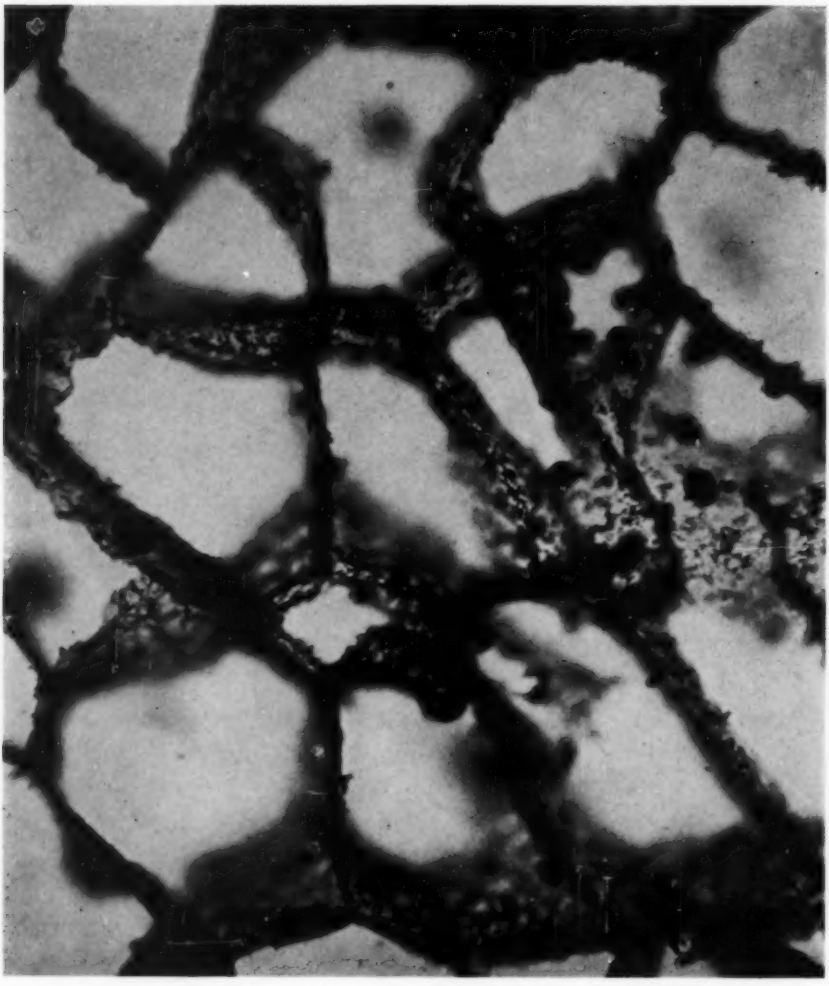

Fig. 10. Portion of cross section of Cuzco corn kernel after a 24-hr 0.4% SO_2 steep. The protein network is largely disintegrated and dispersed; remaining portions have fallen back against the cell walls. Note that the three-dimensional character of the network is clearly evident in this picture. 200 ×.

The apparent sequence of protein hydration, formation of globules, and subsequent progressive disintegration suggests that the formation of the globular bodies represents an early step in the disintegration of

the protein network, followed by breakage into small fragments, with some of the fractions going into ultimate colloidal dispersion.

From the data, it is evident that both high sulfur dioxide concentration and high steeping temperature lead to increased protein disintegration and dispersion. The relationship between protein dispersion and starch recovery is probably due to the fact that the more disperse the protein the less starch it can carry into the tailings or gluten fraction, and, hence, the greater the amount of prime-quality starch left on the table or in the centrifuge. These results on the disintegration of protein by sulfur dioxide are corroborated by the behavior of the gluten fraction from water-steeped corn kept overnight at 49°C in 0.2% sulfur dioxide and in distilled water. In both instances the protein was still in microscopic aggregates, but after the sulfur dioxide steep these were of only about one fourth the diameter of those remaining after the water steep. These partially disintegrated aggregates were found to be birefringent.

In order to determine whether the softening action of sulfurous acid upon the corn kernel might be due to acidity, steeps were run using acetic and hydrochloric acids, respectively, in concentrations equivalent to a 0.2% sulfur dioxide solution. Corn steeped in acetic acid solution was somewhat hard to grind, and separation of starch from gluten was difficult. A 70% recovery of starch, containing 0.10% nitrogen (dry basis), was obtained. After the hydrochloric acid steep, the corn was ground with difficulty. Separation of starch from gluten was fairly easy, but only a 56% recovery of starch, containing 0.07% nitrogen (dry basis), was obtained. The relatively low yield of starch was in part due to the difficulty in grinding the horny endosperm to a finely divided condition. As a result, there were many large particles of horny endosperm screened away from the crude starch.

Similar results were obtained when acetic and hydrochloric acid steeps at pH 1.68 to 1.70, equivalent to that of 0.2% sulfur dioxide, were used. A corn sample steeped only 4 hr in sulfurous acid solution was as easy to process as that steeped for 24 hr in either acetic or hydrochloric acid solution. It was concluded that acidity can play no more than a minor role in softening the corn kernel and loosening the gluten from around the starch granules.

The action of lactic acid as a steeping agent was also studied, since during industrial steeping a considerable amount of this acid is formed by microbial action and is believed by some industrialists to exert a favorable action in the steeping process. Lactic acid in 0.1% concentration effected protein disintegration approximating that brought about by a water steep, and softening roughly equal to that found after a 0.1% sulfur dioxide steep. Both 0.2% and 1% lactic acid steeps

produced protein disintegration about equal to that given by a 0.1% sulfur dioxide steep, and softening approximating that produced by a 0.2% sulfur dioxide steep. A steep containing 0.1% sulfur dioxide and 0.1% lactic acid effected protein disintegration about equal to that resulting from a 0.2% sulfur dioxide steep, but produced slightly more softening than the latter. After the steep containing 0.1% sulfur dioxide and 0.1% lactic acid, the degree of protein disintegration was equal to that produced by a 0.1% sulfur dioxide and 0.2% lactic acid steep, but softening was considerably greater in the latter. Lactic acid has relatively little ability to disintegrate the protein matrix but does exert an appreciable influence in softening the corn kernel. The mechanism of its action is at present inexplicable.

Since the action of sulfur dioxide is not due to its acidity, it might be assumed to be due to its reducing action, especially since sulfites and mercaptans are known to have considerable dispersing action on some proteins. Olcott, Sapirstein, and Blish (1943) have shown that reducing agents have a double action in wheat glutens and flours, first a chemical action on the protein molecule, and second, an activation of the native proteinases present. It is reasonable to expect a similar double effect in corn. The action of reducing agents other than sulfurous acid on the corn protein matrix is under investigation.

Steeping Studies on Artificially Dried Corn. It is common knowledge among corn wet millers that great difficulty is experienced in processing corn artificially dried at 180° to 200°F, apparently because of changes brought about in the endosperm. A sample of corn dried under these conditions was steeped in a 0.2% sulfur dioxide solution for 24 hr at 49°C. The strands in the protein network appeared slightly narrower in the dried corn and upon steeping did not swell to the same extent as those in corn not artificially dried. The starch was, moreover, held more tenaciously by the protein matrix in the artificially dried sample. Protein dispersal during steeping appeared to be nearly normal, except that the disintegrating matrix left pieces which adhered closely to the starch granules. Upon extraction it was impossible to separate all of the gluten from the starch and a prime-quality starch was not obtained.

A second sample of corn, artificially dried at 120°F, appeared intermediate in behavior between corn dried in the field and that dried at 180° to 200°F.

Effect of Steep on Starch. Data showing the effect of the sulfur dioxide concentration of the steep upon noncarbohydrate constituents and viscosity of glutinous and nonglutinous corn starches are summarized in Table III. Nitrogen, or protein, and phosphorus content increased as sulfur dioxide concentration of the steep increased; the

TABLE III

EFFECT OF SULFUR DIOXIDE CONCENTRATION OF STEEP UPON COMPOSITION AND VISCOSITY OF STARCHES

(All steeps were run at 49°C for 24 hr)

Type of corn starch	SO_2 concentration of steep		On vacuum-dried basis				Relative viscosity [1]	
	Initial	Final	N	P	Ash	MeOH extd. fat	Initial	10 min
	%	%	%	%	%	%	*3.5% paste*	
Nonglutinous (Iowa hybrid 939)	0.00	0.00	0.03	0.013	0.06	0.64	7.1	6.1
	0.1	0.05	0.04	0.016	0.06	0.53	5.1	4.0
	0.2	0.1	0.05	0.020	0.05	0.59	4.6	4.5
	0.4	0.2	0.06	0.021	0.06	0.70	3.6	3.2
							2% paste	
Glutinous (Iowax 1)	0.00	0.00	0.03	0.006	0.03	0.20	4.2	2.5
	0.1	0.06	0.03	0.009	0.04	0.16	2.4	2.1
	0.2	0.1	0.05	0.013	0.05	0.20	2.5	2.0
	0.4	0.2	0.08	0.014	0.05	0.24	1.7	1.6

[1] Glutinous starch has a much higher viscosity than nonglutinous; hence, viscosity determinations were made on 2% pastes of the former and 3.5% pastes of the latter. The initial and 10-min values are those obtained immediately after placing the paste in the Stormer viscometer and after the eleventh reading taken at the end of 10 min, respectively.

amount of methanol-extractable fat, however, remained relatively constant. In the nonglutinous corn starch the ash also remained constant, but in the glutinous it increased with increasing sulfur dioxide concentration of the steep.

Starch extracted from either glutinous or nonglutinous corn after a 0.1% sulfur dioxide steep was appreciably lower in viscosity than that extracted after a water steep. The higher the sulfur dioxide concentration of the steep, from 0.1 to 0.4%, the lower was the viscosity of the starch extracted. Viscosity tended to vary inversely with the nitrogen and phosphorus contents of the starches. Sulfur dioxide concentration of the steep was not found to have any appreciable bleaching effect upon the starch.

Summary and Conclusions

A method has been developed for following *in situ* changes occurring in structural protein when corn kernels and, presumably, other cereals are subjected to various conditions. By utilization of this method, it has been found that sulfurous acid in the steep used in the wet-milling process for corn starch extraction has a specific effect in facilitating the separation of starch from protein.

A technique has been developed whereby the starch granules can be

removed from a section of the kernel, leaving the protein network clearly visible. Birefringence of this network indicates that the molecules in it are largely oriented.

In corn kernels the starch in the endosperm cells lies embedded in a proteinaceous matrix. The smaller starch granules and protein predominate in the cells just under the aleurone layer, while larger granules and less protein lie nearer the center of the kernel. In horny endosperm, in general, the protein content is about twice that of the floury endosperm.

Considerable peptization of the protein is effected when corn kernels are subjected to a sulfurous acid steep. At 38° to 43°C, a 0.2% sulfur dioxide steep leaves much of the protein in globules or in strands ending in globules. At 49° to 55°C greater dispersion of the protein results from steeping, and globules are found only in the cells just under the aleurone, where protein packing is most dense. The steep causes more complete protein dispersion, the higher the temperature (38° to 55°C), and the greater the sulfur dioxide content (0 to 0.4%).

Sulfurous acid apparently effects disintegration and dispersal of the protein matrix more by its reducing action than by its acidity. It cannot be successfully substituted by lactic, acetic, or hydrochloric acid in equivalent concentration, or by acetic or hydrochloric acid at the same pH. Addition of lactic acid to a sulfurous acid steep increases softening of the kernel.

Both glutinous and nonglutinous corn starch contain more nitrogen and phosphorus and have lower paste viscosity the higher the sulfur dioxide content of the steep used prior to extraction (from 0 to 0.4%). In glutinous corn starch, the ash content also is greater. The fat content of the starch is not affected by the steep.

It is difficult or impossible to separate prime-quality starch from corn dried artificially at high temperatures, since the protein adheres to the starch granules with unusual tenacity. The difficulty is greater the higher the temperature at which the corn has been dried.

The specific action of sulfurous acid in the steep is to disintegrate the protein matrix throughout the kernel, and, in consequence, to facilitate complete hydration with consequent softening of the kernel. This action is suggested to be due mainly to the reducing action of the sulfurous acid. Bleaching of the starch by the sulfurous acid during steeping is apparently negligible.

Acknowledgments

Corn samples were obtained through the Commodity Development Division of this Laboratory. Analytical data were furnished by the Analytical and Physical Chemical Division. The assistance of Margaret Holzapfel in making starch extractions is gratefully acknowledged.

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EFFECT OF PROTEIN CONTENT AND GRADE ON FARINOGRAMS, EXTENSOGRAMS, AND ALVEOGRAMS [1]

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Machines designed to measure the physical properties of doughs are used in many cereal laboratories. In Great Britain and in continental Europe, the Farinograph and Extensimeter are used extensively for mill control on wheats imported for blending, and it is claimed that the machines provide useful information unobtainable by other means. In Argentina, the government authorities place considerable importance on the physical characteristics of the dough in establishing their standard wheat samples, and reproductions of Farinograms and of Alveograms for different grades are prominently displayed in their crop bulletins. In North America, cereal chemists rely largely on baking tests and subsidiary analyses for evaluating wheat and flour quality, although an increasing interest in dough-testing machines is apparent.

It would perhaps be generally agreed that dough-testing machines, apart from the recording type of dough mixer (Farinograph and Mixograph), have not been investigated widely enough to demonstrate their utility for assessing the quality of North American wheats. In Canada this lack of interest is not surprising, for Canadian wheat owes its position in world markets to its strength, a property that can be evaluated adequately by baking tests. Nevertheless, the widespread use

[1] Paper No. 68 of the Grain Research Laboratory and No. 229 of the Associate Committee on Grain Research (Canada).

of dough-testing machines by importers of Canadian wheat made it advisable for the Board of Grain Commissioners' laboratory to acquire, in addition to the Brabender Farinograph, a Brabender Extensograph and a Chopin Alveograph, and studies of these machines were started some years ago. This paper deals primarily with the effect of protein content and grade on the types of curves produced. The work was done in 1940 with a comparatively small number of samples, and it was the intention to repeat it with a larger number of samples in order that more adequate correlation studies might be made. However, as opportunity for repeating the studies has not presented itself, it now seems advisable to publish the original results.

The influence of protein content on Farinograph curves has been investigated in this and other laboratories. Aitken and Geddes (1938) reported that the dough characteristics measured by the Farinograph are not merely a reflection of protein content; for certain flours of the same protein content and loaf volume gave quite different types of curves. In a later study on gluten-enriched Canadian flours, the same authors (1939) modified this statement when they found that dough-development time and absorption increased with each increment in protein content. Markley (1938), in a study of the dough properties of flour-starch-water systems, also reported that dough-development time is a function of the amount of protein when this is over 7%, but that at lower levels there is no differentiation. Markley and Bailey (1939), in their statistical study of dough formation in an extensive and diverse series of flours, concluded that protein content is highly correlated with dough-development time and with absorption. Geddes, Aitken, and Fisher (1940) showed that dough-development time and curve consistency decreases were, respectively, positively and negatively correlated with protein content; and they also presented considerable data on Farinograms for different grades of Western Canadian wheat.

In an investigation of the Extensograph for predicting baking quality, Munz and Brabender (1940a) mention that there is some relation between protein content and area under the Extensogram. This statement was based on results for "artificial" flours, whose protein contents were either lowered by the addition of starch or raised by enrichment with wet gluten, and doubt was expressed as to whether such flours possessed properties that were identical with those of the original material. The scientific literature does not appear to contain any reference to the effect of protein content on Alveograms.

Investigation of the utility of the Farinograph, the Extensograph, and the Alveograph for evaluating the quality of Western Canadian wheat was begun several years ago, and some of the results obtained

have been referred to briefly in crop bulletins and in the annual reports of the laboratory. As a first step, the machines were used in routine studies of the comparative dough properties of average samples of different grade. Such samples provide a useful test of the machines, for they include wheats that are similar and others that vary widely in both protein content and baking quality. Moreover, their real values are well established and known to both domestic millers and millers in other countries. In general, the results showed that wide differences between certain grades were indicated by all the machines, but it appeared that these differences were closely related to protein content.

To obtain further information on the effect of protein content on Farinograms, Extensograms, and Alveograms, two special sets of samples were collected. These consisted of a series of wheats that varied widely in both protein content and baking strength, and a series of wheats that was essentially the same in these respects. These series, though small, were collected with considerable difficulty and are not easily duplicated. The present paper deals with the results obtained with these two sets of samples and with typical samples representing averages for different grades. The results for each series are discussed in separate sections.

Methods

The Farinograph and Extensograph used in these studies are essentially the same as those described by Bailey (1940) in his comprehensive review of "Physical Tests of Flour Quality." The Alveograph is the redesigned Chopin Extensimeter which he also describes.

Brabender Farinograph. Two types of Farinograms were made, the normal curve and a high-speed curve. The procedure for the normal curve involved the use of a 50-g mixer and was the same as that outlined by Geddes *et al* (1940). Single Farinograms were made for each sample and dough-development time was the only measurement recorded. In this laboratory the standard error of the mean of duplicate curves is of the order of 0.3 min for this measurement.

The high-speed curve was developed by this laboratory in an effort to obtain greater differentiation among samples which usually show little or no differentiation by the normal curve. A 50-g mixer was modified by introducing additional gears so as to increase the speed of the mixing blades 2.3 times. The high-speed curve is made in the same manner as the normal curve, but the weight on the lever system is set in the position recommended for the 300-g mixer. This change is necessary to obtain a curve that falls within the range of the Farinograph scale. The dough consistency level corresponds to 400 Farinograph units. In evaluating high-speed curves, small deviations above

or below the 400 unit line are disregarded. On account of the rapid breakdown of the dough, 10 min mixing time is ample. The curve is interpreted in the same way as the normal curve, but there is a distinct break, and this affords an extra measurement.

The latest model of the Farinograph is equipped with a double-speed mixer; a normal speed of 60 rpm is provided for bread flours, and a slow speed of 30 rpm is provided for soft flours. According to the Brabender Corporation, slow-speed mixing was introduced to bring about gentle development of soft-flour gluten with minimum abuse of the dough. The principle is the same as that employed in this laboratory, but whereas we experimented with a higher speed to obtain faster development of strong flours, they use the reverse to obtain slower development of weak flours.

Brabender Extensograph. Prior to making a test, certain precautions must be taken with the dough mixer, and adjustment of the stretching and recording mechanism may also be necessary; otherwise faulty and misleading curves will be obtained.

The operating instructions supplied with the Extensograph state that all doughs must be mixed in a clean Farinograph mixer after one, and possibly two, cleaning doughs have been mixed and discarded. The explanation given for this is that a very fine layer of copper oxide forms on the surfaces of the mixer overnight, or on long standing, which greatly influences the curves. To determine the minimum number of cleaning doughs that are necessary, and also the time interval between tests when additional cleaning doughs are required, a preliminary study was made using various numbers of cleaning doughs, and with the mixer standing empty for different lengths of time. The results showed that after the mixer stood empty overnight and over a week-end, at least two cleaning doughs are necessary. After a 2-hr interval between mixings, one cleaning dough is required, but after a 4-hr interval two cleaning doughs again seem necessary. For safety, it is the standard practice in this laboratory to employ three cleaning doughs, each mixed for 5 min, before mixing a set of samples for Extensograph tests.

Proper centering of the dough hook is essential, and this can be done by means of the screw situated above it. The dough hook must be positioned in the center of the exposed portion of the dough when it first makes contact with it. If it is slightly to the left, or to the right, the dough may tear on one side when breaking and the length of the curve may be longer than when the dough breaks evenly on both sides.

The necessity for these precautions has been confirmed by Snodgrass [2] in his investigation of the reproducibility of Extensograms. In

[2] Private communications from E. I. Snodgrass, Washington Mills, Glasgow, Scotland.

addition to finding that centering of the dough hook overcame the uneven tearing of the dough when it broke, he observed that several cleaning doughs were required to minimize the effect of the copper poisoning of the mixer. He considers that two cleaning doughs are adequate but additional cleaning doughs are necessary if the time between tests exceeds 5 min.

To conserve paper, and to facilitate comparison of curves, it is advantageous to turn back the kymograph paper on completion of each curve so that replicate curves, and those for each rest period, are superimposed. It is advantageous to start all curves from the same zero mark, and this can be facilitated by installing the mechanism shown in Figure 1 just above the contact that controls the kymograph. A trip

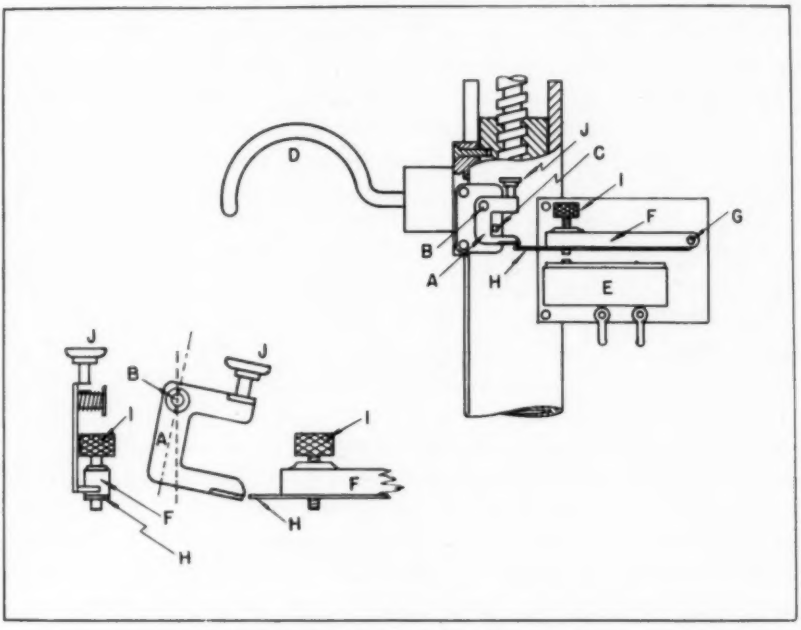

Fig. 1. Drawing of trip switch for Extensograph.

lever *A*, pivoted at *B* and with stop pin *C*, is attached to the dough hook *D*; and a normally closed, snap action, "micro-switch" *E* is attached to the column. The switch is actuated by an arm *F*, pivoted at *G*, and provided with a flexible extension *H* and a screw *I* for making fine adjustments in height. The descending hook is stopped just above the dough when the lever *A* engages the flexible extension *H* on the switch, provided to take care of the dying momentum of the hook after the motor is switched off. By pressing the button *J*, the lever and switch are disengaged (see detail) and the hook starts descending again.

This mechanism automatically stops the hook in the same position each time, and the kymograph paper can be adjusted so that the pen draws duplicate curves from the same starting point.

The Extensograph used in this laboratory has a paper velocity of 6.8 mm per sec and a hook velocity of 14.6 mm per sec. The tension on the balance corresponded to 200 units on the Extensograph paper with a balancing weight of 550 g.[3]

The routine testing procedure is the same as that outlined by Munz and Brabender (1940), with the exception that the absorption of the 300 g of flour is adjusted to 600 instead of 500 Farinograph units, and duplicate curves are made after a rest period of 135 min instead of after 60 min. (In some studies made in this laboratory, curves are also made after a rest period of 45 min.) Two curve measurements are made: maximum height (cm), which measures the resistance of the dough to extension; and maximum length (cm), which measures the extensibility of the dough from moment of first stretching to the final break. The standard error of the mean of duplicate curves is of the order of ± 0.7 cm for an average length of 20 cm, and ± 0.2 cm for an average height of 6.5 cm.

Chopin Alveograph. In this test a fixed absorption is recommended because adjusting the absorption to suit a particular flour frequently causes the curve to run off the kymograph paper. Kent-Jones (1939) states that testing doughs at similar consistencies (variable absorption) does not alter the order of evaluation of flours, though differences between strong and weak flours may be reduced. Although the absorption level varies with different classes of flour, 48% has been found satisfactory for flours milled experimentally from Western Canadian wheat. Dough consisting of 250 g flour (13.5% moisture basis) and sufficient 2.5% sodium chloride solution to bring the absorption to 48% is mixed for 6 min in the "Petrin extracteur." The dough is then extruded and cut into five discs of uniform size. The discs are transferred to a fermentation chamber (25°C) where they remain for 20 min. Each disc of dough, in turn, is then transferred to the Alveograph where it is inflated and the curve recorded. At the moment the bubble ruptures, the volume of the liquid in the gasometer is recorded ("G" value). Three measurements are taken from the mean curve: maximum height (cm), which measures the pressure applied during inflation; maximum length (cm), which measures the extensibility of dough from first application of pressure to the point where the surface of the bubble ruptures; and area under the curve (in square cm). With the aid of the "G" value and the area, a value "W" is computed using the

[3] Since these studies were made, the tension on the balance has been changed to correspond to 200 paper units with a balancing weight of 470 g.

formula: $W = K \times C \times S/L$; where K is a constant (manometer correction coefficient); C, a value associated with the volume of liquid in the gasometer; S, the area under the curve; and L, the length of the curve in millimeters. The value for W is considered to be a quantitative measure of the total work (number of ergs) required to extend the dough. The standard error of the mean of five replicate curves is of the order of $\pm$ 0.4 cm for an average length of 11 cm, and $\pm$ 0.2 cm for an average height of 8.5 cm.

Baking. All loaves were baked by the malt-phosphate-bromate procedure according to the method outlined by Geddes *et al* (1940).

The Variable Protein Series

About 7,000 samples of wheat, of grades 1, 2, and 3 Northern, were collected and analyzed for a protein survey of the 1939 crop of Western Canadian wheat. These were subsequently composited within 0.5% protein ranges to provide a series of samples for the present investigation. A number of the composite samples, at the extreme ends of the protein range, were too small for studies with all machines, but eight samples ranging in protein content from 11.7 to 15.0% were obtained. Annual surveys have shown that in most years 70% of the Western Canadian wheat crop falls within this protein range.

The wheats were milled to yield in a Buhler mill and the resulting flours had the following protein contents and loaf volumes:

Protein content, %	11.1	11.4	12.1	12.4	12.9	13.6	14.1	14.4
Loaf volume, cc	600	635	680	715	765	750	845	875

In any series of composite samples, each representing several hundred cars of grain of essentially the same protein content, all variations in properties except those closely associated with protein content are eliminated. The series then serves admirably for investigations of relations with protein content, but for nothing else. For instance, it will not serve for a study of the relations between Farinogram measurements and loaf volume; for loaf volume is so closely related to protein content ($r = 0.99$) that every property that is correlated with the latter must also be correlated with the former. Nor can this problem be solved by partial correlations; for the variations in loaf volume that are not associated with variations in protein are so small as to provide no scope for partial correlations. Accordingly, it will be profitable with the variable protein series to deal only with the relations between the curve measurements (for Farinograms, Extensograms, and Alveograms) on the one hand, and protein content on the other.

As the curves are more informative than tables of measurements of them, the curves are reproduced in Figure 2. The normal and high-

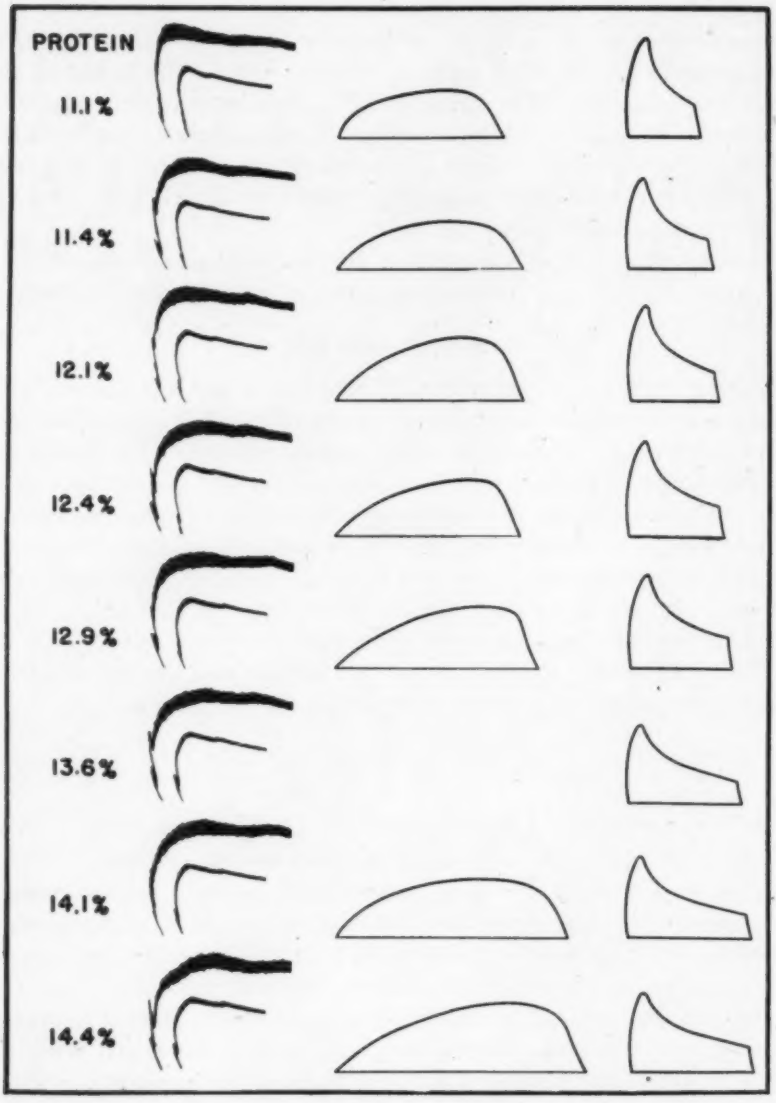

Fig. 2. Farinograms (left), Extensograms (center), and Alveograms (right) for composite samples of increasing protein content.

speed Farinograms are on the left; the Extensograms (135-min rest period) are in the middle; and the Alveograms are on the right.

Farinograms. As the protein increases from 11.1 to 14.4%, the following changes occur in the Farinograms: (1) the time (development time) required to produce maximum dough consistency increases from 2.75 to 7.0 min in the normal curves, and from 1.75 to 3.0 min in the

high-speed curves; (2) the rate at which the dough loses its maximum consistency decreases in both types of curve; and (3) the time (break time) required to bring about the change in consistency that occurs when the dough becomes sticky—shown by a break in the falling curve —increases from 4.0 to 5.75 min in the high-speed curves. In short, with increasing protein content there is a gradual change from a sharply rising curve, which falls off rapidly and shows an early break (more apparent in the high-speed curves), to a curve that rises more slowly and drops not at all or less rapidly.

The closeness of the associations between these changes and changing protein content can be summarized by correlation coefficients; but high correlations must be discounted because there are only eight pairs of values ($n = 6$). The coefficients,[4] listed below, for all Farinogram measurements are all of essentially the same order; the regression coefficients, the average increase in each measurement per 1% increase in protein content, are also listed.

	Correlation coefficient	Regression coefficient
Normal, development time × protein	0.94**	1.13
High-speed, development time × protein	0.95**	0.36
High-speed, break time × protein	0.92**	0.58

These values are considerably lower than the correlation between protein content and loaf volume, $r = 0.99$. In general, the results are in accord with those reported by Geddes *et al* (1940) for development time (normal curve), and by Malloch (1938), who studied the break in curves produced by a recording mixer of his own design.

Extensograms. Figure 2 shows that with increasing protein content the length of the curve, which represents the distance that the dough stretches before it breaks, increases fairly regularly from 16.4 to 25.0 cm; and that the height of the curve, which measures the resistance of the dough to stretching, increases over a narrower range, and less regularly, from 4.6 to 6.6 cm. The curves become much longer and a little higher as protein content increases. Though the area under the curves was not measured, it is obvious from inspection, and from consideration of the data on length and height, that it increases with increasing protein content; the observation of Munz and Brabender (1940a) is thus confirmed.

There are only seven pairs of values for calculating correlation and regression coefficients and this point must be borne in mind in considering the following statistics.

	Correlation coefficient	Regression coefficient
Extensibility × protein	0.96**	2.26
Resistance to extension × protein	0.77*	0.46

[4] Throughout this paper, a single asterisk is used to denote that the correlation coefficients exceed the 5% level of significance, and double asterisks show it exceeds the 1% level.

The correlation for extensibility (length) and protein is of the same order as the Farinogram correlations, but that for resistance to extension (height) is considerably lower.

Alveograms. As protein content increases there is a regular increase in the length of the Alveogram from 7.2 to 12.4 cm, and a regular decrease in height from 10.0 to 7.9 cm. In addition, the work done (as calculated from the formula given previously) increases from 325 to 428 units. The length of the curve represents the time required to burst the bubble, which is inflated under an essentially constant head of water; length is thus a measure of the extensibility of the dough. The height of the Alveogram measures the maximum pressure under the dough just after inflation starts; it appears to be associated with the stiffness, shortness, and tightness of the dough. Thus Alveogram height represents an entirely different property from Extensogram height; indeed this is obvious from the curves, which show that the height of the Alveogram decreases and that of the Extensogram increases with increasing protein content. On the other hand, the lengths of both curves measure essentially the same property, extensibility, albeit under different conditions of extension. On the whole, it appears that in the Alveograms the work done (W) provides the best measure of resistance to extension, the property represented by the height of the Extensogram. Both these measurements increase with increasing protein content.

The correlation and regression coefficients (n = 6) for the Alveogram measurements and protein content are:

	Correlation coefficient	Regression coefficient
Extensibility (length) × protein	0.99**	1.54
Initial stiffness (height) × protein	−0.88**	−0.62
Resistance to extension (W) × protein	0.74*	25.2

The extensibility, as measured by the Alveograph, is apparently more closely related to protein content than any other property measured by this or the other machines; the correlation coefficient is as high as that for protein and loaf volume, though it is by no means certain that this result would be confirmed by a more extensive study. The correlation for protein and resistance to extension is of the same order for the Alveogram as for the Extensogram. It will also be noted that the height of the Alveogram is the only measurement on all types of curves that is inversely related to protein content.

Regression Coefficients. For practical purposes, the regression coefficients that have been listed in the preceding subsections appear to represent the most useful information contained in this paper. Moreover, they should be quite reliable because, though only eight or nine

pairs of values were available for calculating them, the samples represent many subsamples composited to give a series that can vary only in protein content and in properties closely associated with it.

It will have been noted that the changes in the curve measurements for a 1% increase in protein content are quite large: the development time of the normal Farinogram increases by 1.1 min; the length and height of the Extensogram increase by 2.26 cm and 0.46 cm, respectively; Alveogram length increases by 1.54 cm, and height decreases by 0.62 cm. Under these conditions, it is clear that the interpretation of any of these curves for samples of different protein content (such as are found in any normal series of commercial samples) is likely to be based mainly on the effects of the differences in protein content, unless pains are taken to discount them. The regression coefficients are especially useful for this purpose.

The Uniform Protein Series

The samples in this series represented individual parcels of grain shipped from different areas of Western Canada, but it is almost certain that each was a mixture of wheats from different farms. A number of samples of the same protein content were first collected. These were milled and the flours were baked, and the resulting data were used to select samples of flour of essentially uniform protein content giving loaves of about the same size. Nine samples were obtained, and the protein contents and loaf volumes for these were:

Sample	B	I	D	C	A	G	E	F	H
Protein content, %	13.1	13.2	13.2	13.2	13.3	13.3	13.4	13.4	13.5
Loaf volume, cc	745	785	740	750	790	740	755	795	745

The spreads were thus 0.4% for protein content and 55 cc for loaf volume. It should be added that no differences between the handling properties of the doughs could be detected by the baker.

This series makes it possible to examine (1) the ability of the machines to differentiate between samples that cannot be differentiated by the protein determination and the malt-phosphate-bromate baking test, and (2) to examine the relations between the measurements made by the different machines. It will be noted that the second study is equivalent to the calculation of partial correlation coefficients independent of both protein and loaf volume; and it is much more effective and convincing than the calculated partial correlation coefficients.

It was the intention to obtain a series of samples with equal protein content but different loaf volumes, and a series of samples with equal loaf volumes but different protein contents. Studies of these would have been equivalent to calculations of partial coefficients independent

of loaf volume, and of partial coefficients independent of protein content. Unfortunately, it was not possible to collect suitable series of these types.

Differentiation between Samples. The curves for this series, arranged in order of increasing Alveogram length, are shown in Figure 3. Though the spread in protein content was only 0.4%, there is about as much variation in curve type as in the variable protein series which has a spread of 3.3%.

In the normal Farinograms, development time ranges from 4.75 min for sample G to 8 min for sample C; but in the high-speed curves, the spread is only 2.25, *F*, *G*, and *H*, to 2.75, *A*, *B*, *C*, *D*, and *E*. On the other hand, the spread in the break time of the high-speed curves is comparatively large, from 5.25 min for *F* and *I* to 8.0 min for *C*.

Among the Extensograms, the extensibility (length) varies from 19.8 cm for *F* to 23.8 cm for *D*; and the resistance to extension (height) varies from 5.3 cm for *I* to 8.9 cm for *C*.

The Alveograms are no more uniform. Extensibility (length) ranges from 9.7 cm for *A* to 12.6 cm for *I*; stiffness (height) from 7.8 cm for *G*, *H*, and *I* to 10.6 cm for *A*; and work done (W) from 390 units for *F* to 504 units for *D*.

It is thus apparent that all machines were able to demonstrate that differences in physical properties existed between samples that differed little in protein content or in loaf volume obtained with the malt-phosphate-bromate formula.

The machines do not agree entirely on which samples are similar and which dissimilar. That the Farinograph should yield results that differ from those of the other machines is hardly surprising since it operates on an entirely different principle and measures different properties. In development time, the high-speed and normal curves show moderate agreement—possibly as much as could be expected with such little differentiation between high-speed curves. The three samples that have normal curves with the shortest development times are classified as equal and lowest (2.25 min) by the high-speed curves; the four samples that have normal curves with the longest times are classified as equal and high (2.75 min) by the high-speed curves; and of the remaining two curves, which have values of 5.5 min for the normal curves, one is classified as high (2.75 min) and one as intermediate (2.5 min) by the high-speed curves.

The Extensograms and Alveograms might reasonably be expected to classify samples in the same way because they measure similar properties. They did not invariably do so. For instance, on the basis of dough extensibility (length) both machines classify samples *A*, *B*, *C*, *E*, and *H*, in the same manner; but the Extensogram classifies

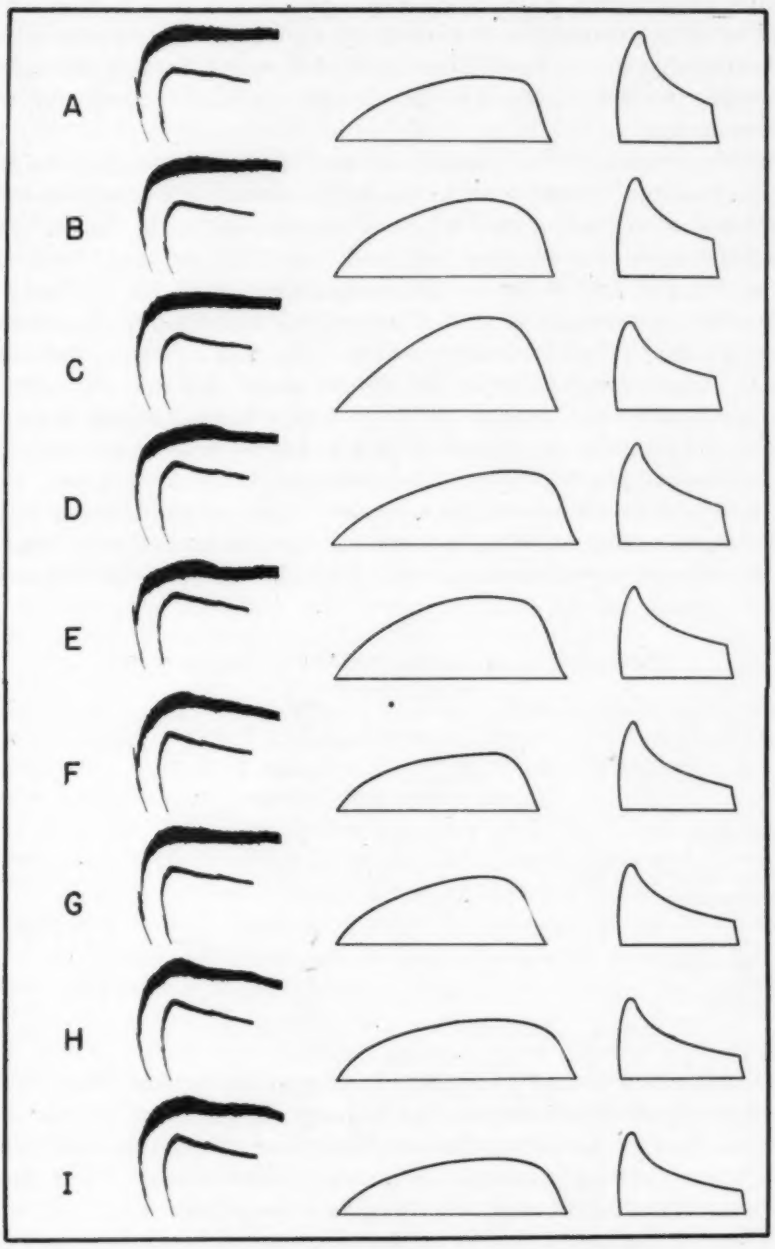

Fig. 3. Farinograms (left), Extensograms (center), and Alveograms (right) for individual samples of essentially the same protein content.

sample *D* as highly extensible and samples *F* and *G* as having little extensibility, whereas the Alveograph classified all three samples in the intermediate range. In addition, sample *I*, which is most extensible according to the Alveograph, is only in the middle range for the Extensograph.

If the height of the Extensogram and the work done (W) by the Alveograph can be considered to measure essentially the same property, resistance to extension, then a further comparison can be made. The machines agree that samples *F*, *H*, and *I* have low values (Alveogram W = 390, 395, and 397 units; Extensogram height = 5.5, 5.8, and 5.3 cm), and that samples *B* amd *E* have fairly high values (Alveogram W = 441 and 447; Extensogram height = 7.4 and 7.9 cm). But sample *C* is classed as highest by the Extensograph (8.9 cm) and only as intermediate by the Alveograph (W = 428), whereas samples *D* and *A* (W = 504 and 482) are classed as highest by the Alveograph and only as intermediate by the Extensograph (height = 7.1 and 6.4 cm).

Relations among Curve Measurements. The relations between the various pairs of curve measurements can best be studied with the aid of the correlation coefficients given in Table I. Those for development

TABLE I

CORRELATION COEFFICIENTS FOR CURVE MEASUREMENTS

Nine samples (n = 7)

Variable	Farinogram, high-speed break time	Extensogram		Alveogram		
		Height	Length	Height	Length	Work
Farinogram						
Normal development time	0.72*	0.80**	0.40	0.34	−0.44	0.59
High-speed break time		0.95**	0.11	0.23	−0.50	0.40
Extensogram						
Height			0.15	0.27	−0.58	0.43
Length				0.07	0.19	0.44
Alveogram						
Height					−0.84	0.88**
Length						−0.69*

time of the high-speed Farinograms have not been included because the machine classified all samples but two into two distinct groups, and there is thus the danger of obtaining significant correlation coefficients which are entirely spurious. All other measurements cover their ranges with sufficient uniform to avoid this pitfall.

The highest correlation is between high-speed break time and the height of the Extensogram. A fundamental relation may well be involved, because break time represents the amount of mixing the dough will stand before it "breaks down" and becomes sticky, and

the height of the Extensogram represents the distance that the dough can be stretched before it breaks; it is thus conceivable that both measurements are related to essentially the same dough property. Both break time and Extensogram height are also related to the development time of the normal Farinogram, *i.e.*, to the amount of mixing required to develop maximum consistency of the dough. It thus appears that all three measurements are controlled, in part, by the same dough property or properties.

The only other significant correlation coefficients involve Alveogram measurements. It is hardly surprising that the work (W) is related to both height and length of the curve because W is a function of the area under the curve, and height and length are involved in this measurement. There is also a high correlation between Alveogram height and Alveogram length, and this is inverse—the greater the height, the less the length. If it is correct that height measures mainly the stiffness and shortness of the dough, and that length measures mainly its extensibility, then the inverse correlation seems reasonable; short, stiff doughs are least extensible. In considering Table I the reader should bear in mind that there were only nine pairs of values for each correlation, and it is therefore quite probable that a larger study would demonstrate additional significant correlations.

There is one other matter of special interest: not only is there no significant correlation between Alveogram length and Extensogram length but the correlation coefficient is the fourth lowest of those listed. This seems surprising since both measurements might reasonably be expected to reflect the extensibility of the dough. It must therefore be assumed that the measurements are made under such widely differing conditions that no correlation between the results is possible.

Several differences between the techniques used with the two machines are readily apparent. First, Extensograms are made with doughs of uniform consistency whereas Alveograms are made at uniform absorption. If the former technique is the better for obtaining a "true" measure of extensibility, then it is easy to understand that the latter technique may give contradictory results with certain samples, and *vice versa*. Second, the Extensograph stretches the dough in only one direction, whereas the Alveograph stretches it in all directions. Third, the Extensograph stretches the dough at a constant rate, which is essentially independent of the resistance of the dough to stretching; whereas the rate at which the Alveograph bubble expands and stretches the dough must be affected by the resistance of the dough to stretching. And fourth, whereas the Extensograph dough is made with water and sufficient salt solution to bring the salt content to 2%, the Alveograph dough is made with 2.5% salt solution and is also

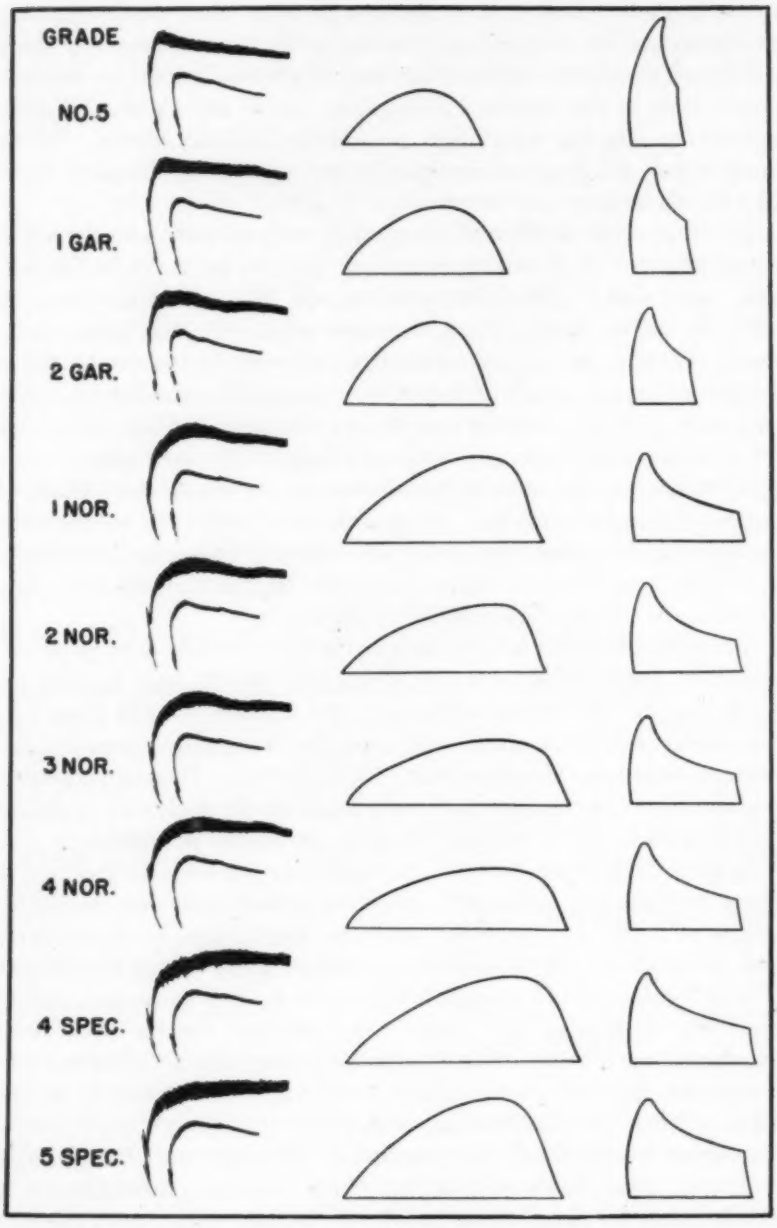

Fig. 4. Farinograms (left), Extensograms (center), and Alveograms (right) for average samples of the grades of the 1939 crop of Western Canadian wheat.

lubricated with oil. In these circumstances perhaps it is too much to expect the two machines to place a series of dough samples in essentially the same rank order with respect to extensibility. Further investigation of this matter is obviously required.

Grade Series for 1939 Crop

Composite samples representing all cars of grain inspected in the Western Division during the 1939–40 crop year were prepared for the grades 1 to 4 Northern, 4 and 5 Special, No. 5, and 1 and 2 C.W. Garnet. Similar samples are prepared each year for certain routine studies, but as 1939–40 was the last crop year in which all these grades were represented, it is fortunate that this investigation was undertaken at that time. The introduction of rust-resistant varieties has made it unlikely that wheat will be graded into the Special grades in future unless there is a severe and widespread drouth. Moreover, during the past few years, the production of Garnet wheat has dropped rapidly and it seems probable that the variety and its grades will disappear entirely within a year or two.

Each of the composite samples represented several million, and some represented many million bushels of wheat. Moreover, the 1939 crop, with a mean protein content of 14.1%, was not far from the long-time average for Western Canadian hard red spring wheats.

Differences among Grades. The curves for the grade series are shown in Figure 4, and the data for protein content, loaf volume, and curve measurements are given in Table II. In both the figure and the table,

TABLE II

PROTEIN CONTENT, LOAF VOLUME, AND CURVE MEASUREMENTS FOR GRADE SERIES, 1939 CROP

Grade	Protein content	Loaf volume	Farinogram			Extensogram		Alveogram		
			Normal	High-speed		Length	Height	Length	Height	Work
			Dev. time	Dev. time	Break time					
	%	cc	min	min	min	cm	cm	cm	cm	units
No. 5	11.1	460	2.0	1.5	5.0	14.1	5.2	5.3	12.6	333
1 Gar.	11.5	525	2.5	2.0	4.5	14.9	8.0	6.0	10.3	320
2 Gar.	11.5	525	3.0	2.0	4.5	16.3	6.4	6.2	9.4	289
1 Nor.	13.1	780	5.5	2.5	5.75	19.8	8.4	11.0	8.3	387
2 Nor.	13.0	765	5.5	2.5	6.0	20.5	6.7	10.9	8.8	410
3 Nor.	13.4	785	5.5	2.5	5.75	22.0	6.4	11.8	8.8	400
4 Nor.	13.6	750	6.0	2.75	5.25	22.1	6.0	11.1	8.7	422
4 Spec.	15.8	885	8.75	3.5	6.0	23.2	8.3	12.3	9.0	499
5 Spec.	16.5	920	10.0	3.5	6.0	21.5	9.7	12.1	10.0	557

the grades are arranged in order of increasing protein content, except that 1 Northern with a protein content of 13.1% has been placed above 2 Northern, which has a protein content of 13.0%.

Figure 4 shows that the curves divide the grades into three groups: No. 5 and the two Garnet grades, the four Northern grades, and the two Special grades. The Farinograms for the first group have short development times and sharp peaks, those for the second group have intermediate development times and rounded peaks, and those for the last group have very long development times and no pronounced peaks. The Extensograms and Alveograms for the first group are short, those for the middle group are intermediate, and those for the last group are long; and the Alveograms for the first and last groups are higher than those for the middle group.

Both protein content and loaf volume classify the samples into exactly the same groups. The values are low for the first group, intermediate for the middle group, and high for the last group. It is thus clear that a large proportion of the differences in curve measurements between grades is caused by differences in protein content. Accordingly, with this series, it will be most profitable to examine each group of grades separately to determine whether grading factors have an effect on curve measurements that is independent of the effect of protein content.

The two Garnet grades have the same protein contents and might be expected to give identical curves. The Farinograms are much the same, except that the normal curve for 2 C.W. Garnet has a slightly longer development time than that for 1 C.W. Garnet. There is a greater difference between the Extensograms; that for 2 C.W. Garnet is shorter and taller than that for 1 C.W. Garnet. Between the Alveograms, there is little difference in length, but that for 1 C.W. Garnet is higher and gives a higher W value than that for 2 C.W. Garnet.

The Northern grades show little difference in the development times of the Farinograms. But, neglecting 1 Northern, there is evidence of a slight decrease in break time with decreasing grade; and this is contrary to expectations based on protein content, which increases with grade. In accordance with the results obtained with the variable protein series, Extensogram height and length would be expected to increase with increasing protein content, *i.e.*, to increase slightly with decreasing grade. The length increases about as much as would be expected, but in 1 to 4 Northern, grading factors reverse the trend for height, which decreases with grade. Among the Alveograms, there are only small differences in length, somewhat larger ones in work, and no evidence of the decreases in height which should occur with increasing protein content. On the whole, the curves for different grades are

very similar. The small differences in protein content are not reflected, but neither is there any clear cut effect of grade.

Although there is a relatively large difference in protein content between the special grades, this is not reflected in all curve measurements. For example, the high-speed Farinograms are identical though there is an increase in the development time of the normal curve. No. 5 Special has a shorter Extensograph length and a greater Alveogram height than No. 4 Special; and these results are contrary to expectations based on protein content.

The 1939 grade series therefore shows that though protein content has a very large effect on curve type, there may be other dough properties, related to grading factors, that affect the curves and tend to upset expectations based on protein content alone. Additional information on this point can be obtained by means of the correlation coefficients given in the following subsection.

Correlation Studies. The pertinent correlation coefficients for the grade series are listed in Table III. Coefficients for the simple correla-

TABLE III

CORRELATION COEFFICIENTS FOR CURVE MEASUREMENTS, PROTEIN CONTENT, AND LOAF VOLUME

Nine pairs of values (n = 7) for simple correlations, and n = 6 for partial correlations

Curve measurements	Simple correlations		Partial correlation [1]
	With protein content	With loaf volume	With loaf volume
Farinogram			
Normal, development time	0.99**	0.95**	0.71*
High-speed, break time	0.78*	0.89**	0.23
Extensogram			
Length	0.83**	0.94**	0.80*
Height	0.68*	0.61	−0.08
Alveogram			
Length	0.87**	0.98**	0.96**
Height	−0.38	−0.68*	−0.98**
Work (W)	0.98**	0.91**	−0.01

[1] Independent of protein content.

tions between curve measurements and protein content are given in the first column of data. Except for that with Alveogram height, all coefficients are significant, though two attain only the 5% level. The high correlations must be partially discounted because there are only nine pairs of values, but it is apparent that most of the differences between grades with respect to dough development time and Alveograph work are associated with differences in protein content. With respect to other measurements, there is evidence of variations that are independent of protein content.

The simple correlations between curve measurements and loaf volume are very interesting. Some are lower than the corresponding correlations with protein content, and some are higher. The latter are the more interesting because they suggest a relation between the curve measurement and loaf volume that is independent of the relation between loaf volume and protein content. The measurements in question are break time, Extensogram length, and length and height of the Alveogram; all these are more closely correlated with loaf volume than with protein content.

In order to examine these relations further it is necessary to calculate the partial correlations between curve measurements and loaf volume, independent of protein content. This procedure gives approximately the same results as would be obtained from a series of samples of the same protein content that differed in curve measurements and loaf volumes. The partial correlation coefficients exceed the 1% level of significance for Alveogram length and height, and the 5% level for development time of the normal Farinogram and for Extensogram length.

Accordingly, these measurements tell something about the loaf volumes that the sample will give that is not told by protein content alone. Among flours of equal protein content, those having longer development times may be expected to give the larger loaves by the malt-phosphate-bromate procedure; those having the greater extensibility, as measured by the length of either the Extensogram or the Alveogram, will give larger loaves; and those that are least stiff (*i.e.*, that give low Alveograms) will give the larger loaves. These results, especially those relating to Alveograms, appear to be quite definite in spite of the small number of samples available for the study.

As a matter of interest, the multiple correlation coefficient for loaf volume on the one hand, and protein content and Alveogram height on the other, was calculated. This turned out to be $R = 0.998^{**}$ and this is significantly higher (1% level) than the corresponding simple correlation between protein content and loaf volume, $r = 0.930^{**}$. Corresponding coefficients for the variable protein curves were $R = 0.997^{**}$ and $r = 0.992^{**}$; and the significance of the added variable exceeded the 5% level. These statistics suggest that it may well be possible to predict loaf volumes more accurately from protein content and Alveogram height than from protein content alone. But, as baking tests can be made more readily than Alveograms, further investigation of this matter with a larger series of samples, though it may be of some theoretical interest, holds little promise of elaborating practical procedures.

Northern Grades Series

The samples in this series represented grades 1, 2, 3, and 4 Northern for each of the five years 1939 to 1943. For 1939, the samples represented all shipments made during the 1939–40 crop year. For the remaining years, the sample for each grade was made up by compositing several hundred individual samples collected all over Western Canada during the first eight or nine weeks after the crop started to move. The composite samples were used to obtain preliminary information on the quality of each new crop, and as the Farinograms, Extensograms, and Alveograms have been reproduced in the annual crop bulletins issued by the laboratory, there is no need to present them again. Data on protein content, loaf volume, and curve dimensions for all samples are given in Table IV.

The data have been exhaustively and repeatedly studied by the authors, both individually and collectively, but to little avail. All conclusions are essentially negative.

No consistent trends can be discovered by considering the differences between grades within years. Because of the relatively small differences in protein content between grades, and keeping in mind the experimental errors, one would hardly expect to discover consistent relations between protein content and curve measurements in these sets of samples. Some effect of grade on curves might be expected if dough properties reflect any of the major grading factors. So far as the authors can determine, there is no evidence among the data for the individual years of a consistent relation between grade and any of the curve dimensions. And this conclusion is certainly supported by the mean data for each grade over all years; for the differences between grades are essentially negligible and by no means consistent.

If the lengths for both Extensograms and Alveograms measure the extensibility of the dough, then in spite of some general agreement there is ample evidence of the ability of the two machines to contradict each other. For instance, in 1940 the Extensograms indicate that 3 Northern is decidedly more extensible than 4 Northern, but the Alveogram indicates that 4 Northern is slightly more extensible than 3 Northern. Again, in 1939 for grades 1 and 4 Northern, and in 1941 for grades 2 and 3 Northern, the Alveograph finds similar extensibilities while the Extensograph finds appreciable differences. And again, in 1942, the Extensograms indicate similar extensibilities for grades 3 and 4 Northern, but the Alveograms suggest a considerable difference between the grades. Until such discrepancies are explained by further investigation, little faith can be placed in data obtained with either machine unless there is some *a priori* reason, of which the authors are not aware, for preferring one to the other.

TABLE IV

PROTEIN CONTENT, LOAF VOLUME, AND CURVE MEASUREMENTS FOR GRADES 1, 2, 3, AND 4 NORTHERN

Year	Grade	Flour protein content	Loaf volume	Farinogram development time	Extensogram 135″ curve		Alveogram		
					Length	Height	Length	Height	"W"
		%	*cc*	*min*	*cm*	*cm*	*cm*	*cm*	*units*
1939	1 Nor.	13.1	780	5.5	19.8	8.4	11.0	8.3	387
	2 Nor.	13.0	765	5.5	20.5	6.7	10.9	8.8	410
	3 Nor.	13.4	785	5.5	22.0	6.4	10.8	8.8	400
	4 Nor.	13.6	750	6.0	22.1	6.0	11.1	8.7	422
1940	1 Nor.	13.4	785	5.0	18.7	5.0	9.0	9.5	353
	2 Nor.	13.6	780	5.5	19.3	4.8	9.6	9.7	385
	3 Nor.	13.9	820	5.25	19.2	4.5	11.0	8.5	312
	4 Nor.	13.6	750	5.0	13.5	6.4	11.9	8.3	289
1941	1 Nor.	13.8	815	6.5	20.7	7.4	10.5	8.6	448
	2 Nor.	14.2	845	7.0	19.6	7.4	11.5	9.0	494
	3 Nor.	14.1	840	7.0	22.5	7.8	11.2	9.0	507
	4 Nor.	14.4	900	6.0	24.5	7.8	12.3	8.0	491
1942	1 Nor.	12.1	710	5.5	17.4	7.6	8.1	9.2	383
	2 Nor.	12.0	705	4.5	17.0	7.2	9.4	9.3	417
	3 Nor.	12.1	740	5.5	15.6	6.8	9.4	9.2	429
	4 Nor.	12.0	720	5.0	15.6	6.5	7.6	9.8	392
1943	1 Nor.	13.3	795	4.5	19.4	5.4	11.5	7.7	383
	2 Nor.	13.1	795	4.5	19.4	5.0	11.1	7.7	415
	3 Nor.	12.6	775	4.0	18.8	4.2	9.8	7.9	349
	4 Nor.	12.6	770	3.5	17.5	3.5	8.3	8.5	339
All years	1 Nor.	13.1	775	5.5	19.2	6.8	10.0	8.7	391
	2 Nor.	13.2	780	5.5	19.2	6.2	10.5	8.9	424
	3 Nor.	13.2	790	5.5	19.6	5.9	10.4	8.7	399
	4 Nor.	13.2	780	5.0	18.6	6.0	10.2	8.7	387
1941	All	14.1	850	6.5	21.8	7.6	11.4	8.6	485
1940	grades	13.6	785	5.25	17.7	5.2	10.4	9.0	335
1939		13.3	770	5.5	21.1	6.9	11.0	8.6	405
1943		12.9	785	4.0	18.8	4.5	10.2	8.0	372
1942		12.0	720	5.0	16.4	7.0	8.6	9.4	405

When the average differences between crops are considered with the aid of the means for all grades, some general trends can be discerned. Part, but by no means all, of the differences in curve measurements between crops can be traced to differences in protein content. Moreover, when the five crops are placed in order with respect to protein content, the 1940 crop is consistently out of order, and in the right direction, for all curve measurements. In addition, the 1943 crop, which is out of order for development time, is also out of order for Extensogram height and Alveogram "W," and for Alveogram height, though in the wrong direction. There is also some agreement between extensibility measurements by both the Extensograph and the Alveo-

graph; both machines place 1941 first, 1939 second, and 1942 last with respect to length. But these are the three crops on which all machines and measurements agree in placing the crops in order of their protein contents. Thus, on the whole, the mean values for each crop are not particularly illuminating.

The series under discussion certainly fails to provide any evidence that grade and factors associated with it bear any relation to the dough properties measured by the machines. This, of course, is no disparagement; for protein content and loaf volume, both of which are useful criteria of wheat quality, are not related to grade either.

Summary

Four series of samples of Western Canadian wheat were employed in a study of the effect of protein content and grade on Farinograms, Extensograms, and Alveograms. Farinograms made with a high speed mixer (2.3 times normal speed) were also studied. These provided an additional measure, break time; this represents the time that elapses before the dough "breaks down" and becomes sticky, which causes a sharp break in the Farinogram.

The first series consisted of eight composite samples ranging in protein content (flour) from 11.4 to 14.4%. All curve dimensions were directly correlated with protein, except Alveogram height with which the correlation was inverse. The regression coefficients of the principal curve dimensions on protein content were: dough-development time of Farinogram 1.1 min; Extensogram length and height, 2.26 and 0.46 cm; and Alveogram length and height, 1.54 and −0.62 cm, per 1% increase in protein content.

The second series consisted of nine individual samples of essentially the same protein content and baking strength. All machines demonstrated that differences in dough properties existed between the samples; but even the Extensograph and Alveograph, which appear to measure much the same dough properties, did not always agree on which samples were similar and which dissimilar. Moreover, no correlation was apparent between Extensogram length and Alveogram length, both of which measure the extensibility of the dough—though under different conditions. The highest correlation was obtained between break time and Extensogram height ($r = 0.95$). Extensogram height was also correlated with development time of the normal Farinogram ($r = 0.80$), and development time was correlated with break time ($r = 0.72$). No other correlations between dimensions of curves from different machines were significant.

The third series consisted of composite samples of nine grades of the 1939 crop. All machines divided them into three groups: No. 5

and 1 and 2 C.W. Garnet; 1, 2, 3, and 4 Northern; and 4 and 5 Special. But determinations of protein content and baking strength (malt-phosphate-bromate) divided the series into exactly the same groups; group 1 was low in protein content and loaf volume, group 2 was intermediate, and group 3 was high. Within each group, there were slight indications that some dough properties were related to grading factors and this had a tendency to upset expectations based on protein content alone. Several curve dimensions were closely correlated with both protein content and loaf volume, and the partial correlations for Alveogram length ($r = 0.96$) and for Alveogram height ($r = 0.98$) and loaf volume, independent of protein content, exceeded the 1% level of significance.

The fourth series consisted of average samples of grades 1, 2, 3, and 4 Northern for the five years 1939 to 1943. The data for this series were anomalous, and certainly failed to demonstrate that any of the curve dimensions tend to change uniformly with decreasing grade.

Acknowledgments

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REPRODUCIBILITY STUDIES AND SOME EFFECTS OF TECHNIQUE ON EXTENSOGRAMS AND ALVEOGRAMS [1]

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In a previous paper, Aitken, Fisher, and Anderson (1944) described the effects of the protein content and grade of wheat on Farinograms, Extensograms, and Alveograms. Before undertaking further work of that sort, it seemed desirable to make a more comprehensive study of the reproducibility of the curves, and to examine the effects of certain adjustments of the Extensograph, and of a variation in the procedure used with the Alveograph. The same flours were used with each machine, but in other respects the investigations were quite separate. Accordingly, this paper is divided into two sections dealing respectively with the Extensograph studies and the Alveograph studies.

Extensograph Studies

With the Extensograph, the resistance of the dough to extension is measured by the pull that the dough exerts on one arm of a balance. The downward movement of the balance arm measures the force applied by the dough. As the movement is quite small, it is converted by a system of levers into a much larger movement of a pointer, which is equipped with a pen that writes on a kymograph. The increasing resistance of the dough to extension as it is stretched is thus recorded as increasing height of the Extensogram.

Weak and strong flours have widely different resistances to extension, and the lever system on the Extensograph is therefore made adjustable so that the machine can be used with both types of flour. At a suitable setting of the lever system, the pen responds readily to the comparatively small pulls exerted by weak flours, but if strong flours are used with this setting the curves rise rapidly and run off the kymograph paper. By adjusting the lever system to make the pen less responsive, curves for strong flours can be kept on the paper but those for weak flours hardly rise at all.

At one time or another, three different settings have been used in this laboratory, and it seemed desirable to obtain information about the relations between the curves obtained at these settings, and about the

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effects of setting on the sensitivity of the machine and reproducibility of its curves. One of the settings is widely used in Europe, and the second and third are both used on this continent.

Specification for Settings. The settings are specified by the movement of the kymograph pen for given loads on the dough holder. As the arm which supports the dough holder is part of the lever system, the load must be suspended at the same distance from the fulcrum as is the center of gravity of the dough when under test. The movements of the pen are recorded in the units represented by the lines on the kymograph paper, or in centimeters, which is the practice in this laboratory. By careful adjustment of the lever system a straight-line relation can be obtained between the load and the position of the pen on the paper. This point is illustrated by Figure 1, which shows the rela-

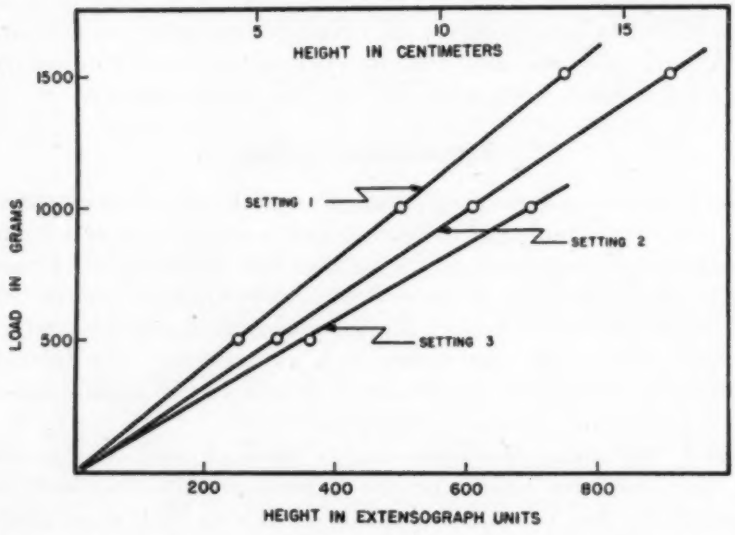

Fig. 1. Curves showing the relation between the load on the dough balance of the Extensograph and the height registered by the pen for three settings of the machine.

tions between load and pen for the three settings which were investigated.

It follows that laboratories using different settings of their Extensographs should be able to report resistance to extensibility (height of curves) on the same scale by converting the units on the kymograph paper to grams by means of an appropriate factor. Alternately, the height can be recorded in centimeters and a factor for converting this to grams can be computed. The factors for the three settings shown in

Figure 1 are 2.00, 1.63, and 1.41 for kymograph units, and 112.0, 91.5, and 79.0 for centimeters.

Plan of Investigation. The three flours chosen for this study were experimentally milled from (A) a 1 Northern wheat, (B) a No. 5 wheat, and (C) a 1 C.W. Garnet wheat. They had protein contents of 12.8, 13.5, and 11.1%.

The general technique employed with the Extensograph in this laboratory has been described by Aitken, Fisher, and Anderson (1944). The curves discussed in the present paper were made after a 45-min and 135-min rest period.

For each flour, duplicate 45-min and duplicate 135-min curves were made from the same doughs, in the morning and in the afternoon, on each of four different days during which one setting was maintained. This procedure was then repeated with each of the other settings. A factorial design was thus used, with three flours, three settings, eight replications (mornings and afternoons of four days), and duplicate curves.

Results and Discussion. The effects of the different settings on the shapes of the curves are of first interest. Figure 2 shows average

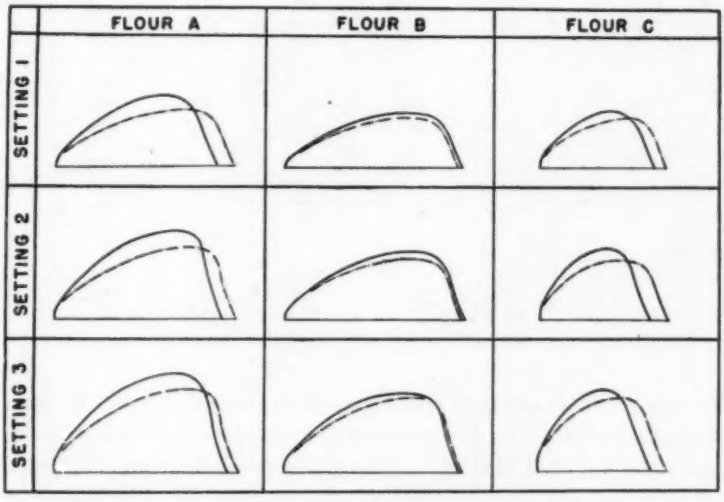

Fig. 2. Extensograms (45 min, dotted; 135 min, solid) for three settings of the machine.

curves for each of the three flours at each of the three settings, and data on length and maximum height are given in Table I. Inspection of the data indicates that the changes in the relative heights and lengths of the curves at different settings are essentially negligible, and statistical

analyses confirmed this conclusion. With settings representing in-increasing sensitivity, the length of the curve (extensibility of the dough) is not affected and the height of the curve increases. If the latter measurement, which represents resistance to extension, is translated into grams, most of the variation is removed. For each flour the data for all three settings agree within about ±2%. Better agreement might possibly be obtained by more precise adjustment and calibration of the machine at each setting. However, it is clear that if different laboratories, using different settings for their Extensographs, would report resistance to extension in grams rather than in kymograph units or centimeters, the results could be more readily compared.

TABLE I

CURVE MEASUREMENTS FOR THREE FLOURS AT THREE EXTENSOGRAPH SETTINGS

Flour	Setting No.	Length		Height			
				As measured		Converted to grams	
		45-min	135-min	45-min	135-min	45-min	135-min
		cm	*cm*	*cm*	*cm*	*g*	*g*
A	1	20.5	18.5	6.6	8.1	740	910
	2	20.5	19.0	8.1	10.1	740	920
	3	20.7	19.5	9.8	11.3	770	890
B	1	20.0	20.4	5.7	6.3	640	710
	2	20.4	20.5	7.2	8.0	660	730
	3	20.5	20.1	8.7	9.2	690	730
C	1	14.4	13.1	5.7	6.5	640	730
	2	14.9	12.8	7.1	8.4	650	770
	3	14.9	13.0	8.5	9.6	670	760
Mean, all flours	1	18.3	17.4	6.0	7.0	670	780
	2	18.6	17.4	7.4	8.8	680	810
	3	18.7	17.6	9.0	10.0	710	790

The question of whether the flours were placed in exactly the same relative positions by the 45-min and the 135-min curves can be examined with the aid of Table II. There was no change in the length for flour *B* between the 45-min and 135-min curves, but lengths for flours *A* and *C* shortened appreciably; moreover, the order for flours *A* and *B* was reversed from one curve to the other. Again, for height as measured or converted, the order for flours *B* and *C* was reversed. It is thus clear that the rank order for certain flours will be affected by the length of the rest period between mixing and stretching. This is an interesting point because there appears to be no *a priori* reason for

TABLE II

MEAN VALUES FOR EACH FLOUR OVER ALL SETTINGS FOR 45-MIN AND 135-MIN CURVES

Flour	Length		Height			
			As measured		Converted to grams	
	45-min	135-min	45-min	135-min	45-min	135-min
	cm	*cm*	*cm*	*cm*	*g*	*g*
A	20.6	19.0	8.2	9.8	750	910
B	20.3	20.3	7.2	7.8	660	720
C	14.7	13.0	7.1	8.2	650	750

selecting one rest period rather than another. Whether it is worth while as a routine procedure to use two rest periods and examine the changes that occur from one to the other with different flours has not yet been established.

The differences that can occur between curves made from the same flour by a standard method are illustrated in Figure 3. This shows

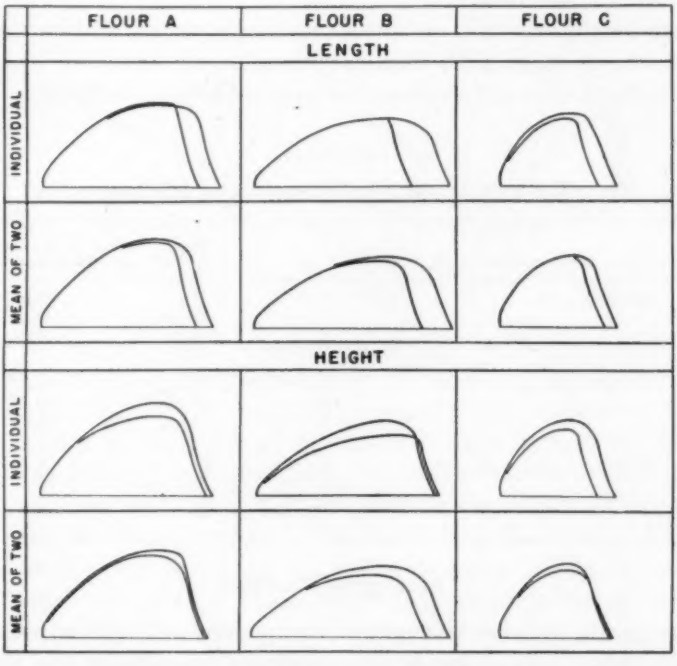

Fig. 3. Extensograms (135 min) showing the range in length and height obtained with individual curves, and with the mean curves (mean of 2 curves from one dough) for eight replicate doughs.

135-min curves for setting 2. Sixteen curves (2 for each of eight doughs) were made for flour *A*, and of these the longest and shortest are shown in the top left-hand corner of Figure 3. A similar selection from the eight curves representing means of two curves made from the same dough are shown immediately below. Corresponding pairs of curves for flours *B* and *C*, and the corresponding sets of curves for height, make up the rest of the figure. In drawing conclusions about the reproducibility of Extensograms, from this figure, the reader should bear in mind that the curves represent the maximum spreads obtained in the investigation.

Statistical analyses of the curve measurements showed that the differences between curves for doughs mixed on different days were not significantly greater than those between doughs mixed on the same day; all eight doughs from one flour can therefore be treated as samples from one population. Comparison of the inter-dough and intra-dough errors showed that the former were significantly greater for height at settings 1 and 3, and for length at setting 2. Although these results are not particularly clear cut, they indicate that in comparing two flours a better estimate of error will be obtained if single curves are made for duplicate doughs from each flour, in preference to duplicate curves for a single dough.

Data for the standard deviations for single curves, and for the mean of two curves made with duplicate doughs, are listed in Table III. These statistics are self-explanatory and require no comment.

TABLE III

STANDARD DEVIATIONS FOR EXTENSOGRAM MEASUREMENTS

Measurement	Setting	Single curve	Mean of two curves from different doughs
Length, cm	1	0.92	0.65
	2	0.98	0.69
	3	0.93	0.66
Height, cm	1	0.73	0.52
	2	0.36	0.25
	3	0.68	0.48
Height, converted to g	1	82	58
	2	33	23
	3	54	38

Alveograph Studies

The dough for the Alveograph is prepared in a special mixer, the Petrin. After mixing for 6 min, a slit in the side of the Petrin is opened by raising a slide. The continued operation of the mixer arm

then forces the dough out through the slit in a strip about 5 cm wide by 0.5 cm thick. As the strip is extruded, 5 to 6 cm lengths are cut off; these are rolled to standard thickness, and a disc (diam., 4.5 cm) is cut from each with a cylindrical cutter. The discs are put into a conditioning cabinet and after 20 min they are inflated on the Alveograph. An interval of about 1 min elapses between the inflation of successive discs, of which there are five for each dough.

Inspection of the quintuplet curves showed that the last tended to be the highest and shortest and that there were certain other consistent differences. The variations in the curves make it difficult to draw the mean curve, and it is desirable to reduce the variation. It appeared

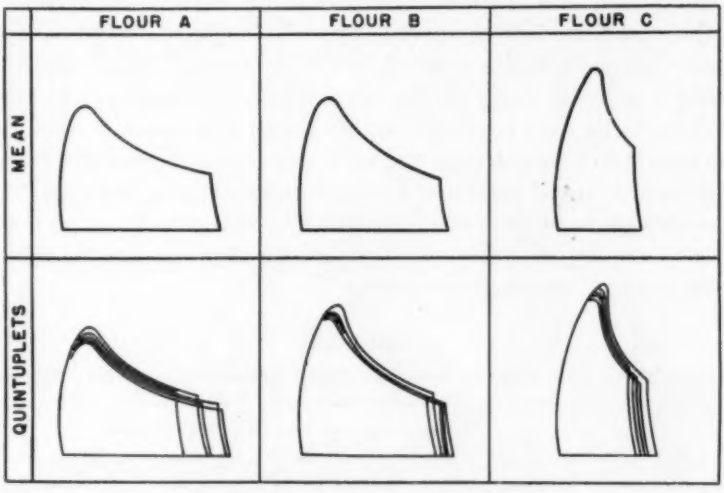

Fig. 4. Mean Alveograms (of quintuplet curves from eight doughs) for three flours, and representative groups of quintuplet curves for single doughs.

that it was caused by the longer mixing to which the last disc was exposed, or possibly to differences in the pressure at which the strip was extruded from first to last. But to test this hypothesis it was necessary to inflate the discs in reverse order so as to investigate the effect of the time factor. A study of reproducibility was also undertaken.

Plan of Investigation. The investigation was closely similar to that made with the Extensograph. The same three flours were used, and eight doughs were mixed, on the morning and afternoon of four different days. In place of the three Extensograph settings, two techniques, normal and reverse order of inflating the discs, were studied; and instead of duplicate Extensograms, quintuplicate Alveograms were made.

Results and Discussion. The mean curves for each flour, obtained by the normal order of testing, are shown in the upper half of Figure 4, and in the lower half are shown representative groups of curves for the quintuplet discs from one dough of each flour. The latter curves illustrate the type of variation obtained with the Alveograph.

Mean data for each disc, over all flours and replicates, are given for both orders in Table IV. These data show that the reverse order of testing did not change the rank order of the discs; the last disc tended to give the highest and shortest curve irrespective of whether it was inflated first (normal order) or last (reverse order). On the average, the length of the curve decreased from disc 2 to disc 5 in both normal and reverse procedures. There was also an increase in height from disc 3 to disc 5 in the reverse order; but in the normal order the third disc gave the lowest curve. Spreads between curves were considerably larger for the reverse than for the normal order, and this is certainly a point in favor of the normal order of testing. It is clear that, though the time between extrusion and inflation has some effect on the quintuplet curves from the same dough, the differences between the curves are caused principally by differences in the properties of the consecutive parts of the extruded dough. Accordingly, it seems reasonable to suggest that the whole technique of preparing the discs for inflation requires thorough revision.

TABLE IV

MEAN DATA FOR NORMAL AND REVERSE ORDERS OF INFLATING DISCS

Disc No.	Length		Height	
	Normal	Reverse	Normal	Reverse
	cm	*cm*	*cm*	*cm*
1	9.3	9.2	10.0	9.8
2	9.3	9.2	9.8	9.8
3	8.8	8.7	9.7	9.8
4	8.4	8.1	9.9	10.2
5	8.2	8.0	10.5	11.0
Spread	1.1	1.2	0.8	1.2

As it is apparent that the normal order of testing is superior to the reverse order, the following discussion is confined to results obtained with the normal order.

The quintuplet curves can hardly be considered satisfactory replicates, and it seemed possible that an advantage might be gained by discarding three discs and considering the remaining two as duplicates. The data in Table IV suggested that the first two discs would prove most satisfactory, and inspection of the raw data confirmed this con-

clusion. A statistical comparison of the results of using the first two discs and all five discs showed that no real advantage could be gained. As would be expected, the standard deviation of measurements on a single curve from one dough decreased appreciably when only the first two discs were considered. But this was offset by a loss—with the majority of flours and measurements—in the precision of the mean data for one dough; the standard deviation for the mean of five discs for one dough tended to be lower than the standard deviation of the mean of the first two discs for one dough.

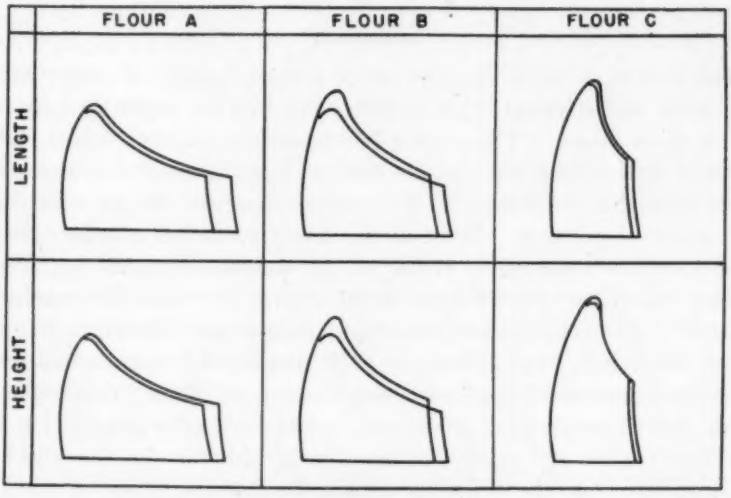

Fig. 5. Mean Alveograms (of quintuplet curves) showing the range in length and height obtained with eight replicate doughs.

The curves for the five discs made for one dough cannot be treated as replicates, and a discussion of the replicability must therefore be confined to that of the mean curve for separate doughs. Statistical analyses demonstrated that the differences between doughs examined on different days were no greater than those between doughs examined on the same day (with one negligible exception), and the eight doughs for each flour were therefore treated as representatives of one population.

Experimental errors of the mean curves for single doughs are illustrated in Figure 5, which contains a series of curves showing the maximum differences in height and length encountered with each flour. Corresponding data for the standard deviations of the mean curves are listed in Table V. The statistics show that a useful level of reproducibility is attained.

TABLE V

STANDARD DEVIATIONS FOR MEAN ALVEOGRAM MEASUREMENTS FOR ONE DOUGH

Measurement	Flour	Mean value	Standard deviation
		cm	*cm*
Length	A	11.0	0.4
	B	9.7	0.3
	C	5.7	0.2
Height	A	8.7	0.2
	B	9.8	0.3
	C	11.4	0.1

Summary

The effects of three settings of the Extensograph on curve dimensions were investigated with duplicate curves for eight doughs from each of three flours. The setting has no effect on curve length, which measures the extensibility of the dough; but the height of the curve, which measures resistance to extension, increases as the machine is made more responsive. Most of the latter variation can be removed if resistance is reported in terms of the weight in grams required to produce the given curve height at the setting at which the machine is adjusted. The relation between curve height and resistance in grams can be made essentially linear at each setting. The standard deviations of measurements made on a single curve are of the following order: length, ±0.95 cm; height, ±0.60 cm; and resistance in grams, ±0.55 g.

When curves are made on the Alveograph for the five discs cut consecutively from the extruded dough, the last disc gives, on the average, the highest and shortest curve. A study made with eight doughs from each of three flours shows that this happens whether the discs are inflated in normal or reverse order. The data show that though the time between extrusion and inflation has some effect on the quintuplet curves, the differences between these are caused principally by differences in the properties of the consecutive parts of the extruded dough strip. The quintuplet curves cannot be treated as replicates; replication can only be obtained by working with two separate doughs. It appears that the technique of preparing discs for the Alveograph requires thorough revision. The standard deviations for the mean of five curves made from a single dough are of the following order: length, ±0.3 cm; and height, ±0.2 cm.

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SUSCEPTIBILITY OF BISCUITS TO INSECT DAMAGE [1]

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Hard biscuits of the type issued to the armed forces for emergency rations must frequently be stored for long periods, during which insect infestation may be an important cause of spoilage. The extent of such spoilage will be determined largely by the suitability of the biscuits for insect development and survival, and the development and survival of insects will be affected by the moisture content and the ingredients of the biscuits. If these factors are favorable to insects, then extensive damage may occur; and to the extent that they are unfavorable, the biscuits will be resistant to insect attack.

This study was designed to test the susceptibility of biscuits to insect damage when the moisture content and the principal biscuit ingredients were varied.

Before initiating the insect study it was necessary to determine the moisture equilibrium values for biscuits at different relative humidities. These determinations are reported in the first part of the paper; the second part reports experiments in which the confused flour beetle, *Tribolium confusum* Duv., was cultured on biscuits of varied composition and moisture content.

Effect of Flour Grade and Fat Level on Moisture Equilibrium Values

The moisture content of dried foods is frequently a limiting factor for the development of insects feeding on them. Consequently, the effect of the principal constituents of the biscuits on the moisture equilibrium values at different relative humidities was given careful study.

Flour and fat are two important components in a typical ration biscuit formula and they are used in relatively high proportions; the former is the main basic ingredient and there is a tendency to use maximum amounts of the latter to increase the caloric value. Flour is hygroscopic and fat is not, and it is thus apparent that the proportions used will have an effect on the hydration capacity of the biscuits.

This section of the study deals with the moisture equilibrium values of biscuits processed from three grades of flour and three levels of fat when stored at three levels of relative humidity.

Materials and Methods. Two flours, one of 50% extraction and one

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of 85% extraction, were milled from a typical soft wheat of 10.2% protein content. A blend of equal parts of these flours provided a third flour of intermediate extraction. The protein and ash contents were 8.5% and 0.39% for the short-extraction flour, 9.0% and 0.66% for the blend flour, and 9.6% and 0.92% for the long-extraction flour.

The formula for a single batch of six biscuits was: flour, 100 g (13.5% moisture basis); cane sugar, 5 g; milk powder, 8 g; baking soda, 1.5 g; salt, 1.5 g; fat (hydrogenated peanut oil), variable; and distilled water, 38 ml. The fat levels were 2, 7, and 12 g. Biscuits were made by mixing all ingredients in a single-arm low-speed mixer (63 rpm) for 4 min, passing the dough through sheeting rolls three times, shaping with a cookie-cutter, and pricking the surface of the dough with a fork. The biscuits were baked on a wire-mesh mat for 12 min in an oven maintained at 230°C. This procedure gave an oval-shaped biscuit with dimensions of about 45 × 40 × 5 mm. Each batch of biscuits was cooled to room temperature and set aside in a closed container until the total number required was obtained.

Three desiccators, maintained at 25, 50, and 75% relative humidities by means of appropriate concentrations of sulfuric acid, were used as storage chambers. Each desiccator was equipped with three tiers of glass shelving, to support the moisture tins containing the biscuits, and a small two-bladed fan to circulate the air. The fan-shafts passed through a mercury-seal in the stopper of each desiccator and the three fans were propelled by a low-speed motor. The desiccators were maintained at a controlled temperature of 27°C. The initial moisture contents of the biscuits were determined by the A.O.A.C. vacuum oven method using random samples ground in a Wiley mill ($\frac{1}{2}$ mm mesh sieve).

To determine the rates of moisture increase and the final moisture equilibrium values, triplicate samples of whole biscuits made from each of the nine formulae (three grades of flour and three levels of fat) were exposed to each of the three levels of humidity and weighed at intervals until constant weights were obtained. For the first few days, when the moisture increase was quite rapid, the weighing interval was 24 hr; later, the interval was extended to 48 hr or longer. The data were tabulated in terms of moisture content at each weighing, the values being computed from the original dry weight of each biscuit. As a check on the calculated equilibrium values, the final moisture contents of the biscuits were determined by the vacuum oven method.

To test the effect of aging, some of the biscuits were set aside in sealed tins at room temperature for a period of six months. The hydration capacity of these biscuits at the three levels of relative humidity was then determined in the same way as for fresh biscuits.

Experimental Results. The mean moisture data showing the moisture content of the fresh biscuits after exposure at the different humidities are summarized graphically in Figure 1 which shows the mean effect of fat level over all flours on the rate of moisture increase and on the equilibrium values, and in Figure 2 which shows similar comparisons for each flour over all fat levels.

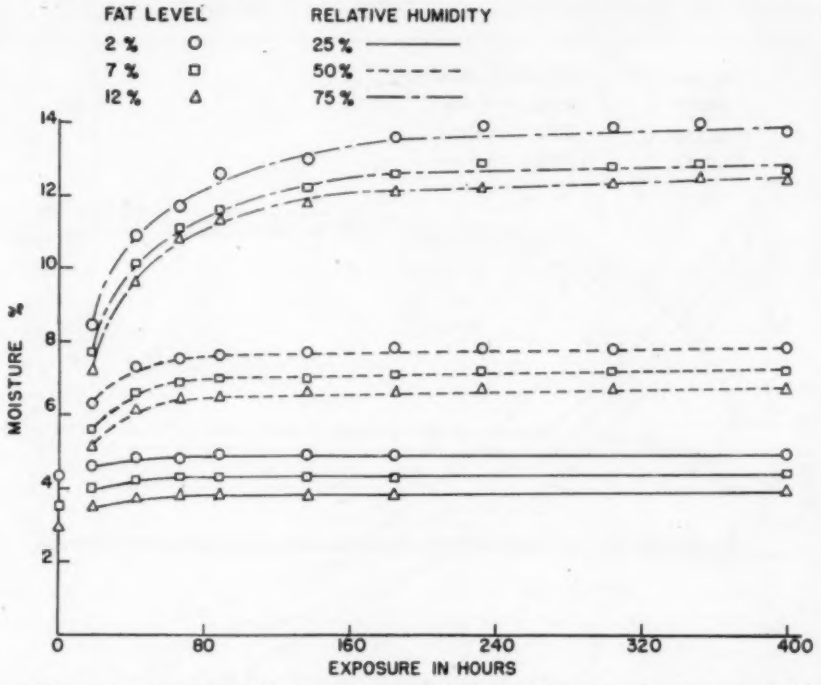

Fig. 1. The mean effect of fat content, over all flours, on the rate of moisture increase and the equilibrium moisture content of biscuits. The initial moisture contents of the biscuits are indicated by the points on the ordinate.

The moisture content of the biscuits increased quite rapidly during early exposure and then showed a drift to equilibrium. As the difference between the initial and final moisture content increased with each increase in relative humidity, the trend is obviously most apparent at the 75% humidity level. As would also be expected, equilibrium was attained more rapidly and at a lower level with each decrease in humidity.

The effect of increasing the fat content is clearly to lower both the initial moisture content and the equilibrium moisture content, at all levels of relative humidity. This effect is probably due to the displacement of the hygroscopic material, flour, by the nonhygroscopic material, fat. It is further apparent from Figures 1 and 2 that fat

content exerted a greater influence on moisture content than did flour grade. For example, the biscuits made from the long-extraction flour exposed to 75% humidity attained an equilibrium of 13.7% moisture for the 2% fat level, as compared with 12.0% moisture for the 12% fat level. At the 12% fat level, the corresponding values for the short-extraction and long-extraction flour biscuits were 12.8% and 12.0% respectively. Thus the spread in moisture content induced by the fat variable was more than twice that induced by the flour variable.

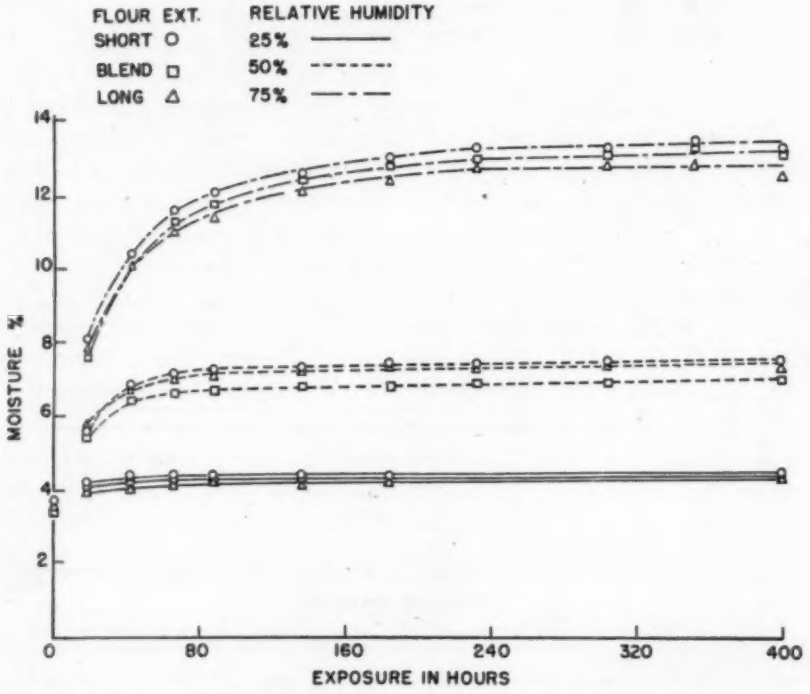

Fig. 2. The mean effect of flour extraction, over all fat levels, on the rate of moisture increase and the equilibrium moisture content of biscuits. The initial moisture contents of the biscuits are indicated by the points on the ordinate.

Figure 3 compares the hydration capacity of fresh biscuits at two levels of fat content with similar values for biscuits six months old. At relative humidities of 50% and 75% the hydration capacity of aged biscuits is reduced as compared to fresh biscuits, while at 25% relative humidity it is increased somewhat by aging. The effect of fat content remains the same for aged biscuits as for fresh biscuits. Typical values for fresh and aged biscuits (2% fat level) are: 4.9% and 5.6% at 25% relative humidity; 8.0% and 7.4% at 50% relative humidity; and 13.7% and 12.3% at 75% relative humidity.

Effect of Moisture Content, Fat Level, and Flour Grade on Insects

A reliable measure of the susceptibility of a foodstuff to insect damage is the rate of development of insects feeding on it. Another measure is the percentage survival of the insects. Both measures relate to the fact that the damage caused by insects is a function of their numbers; and the number of insects also determines the possible extension of the range of infestation. Thus, insects gaining access to

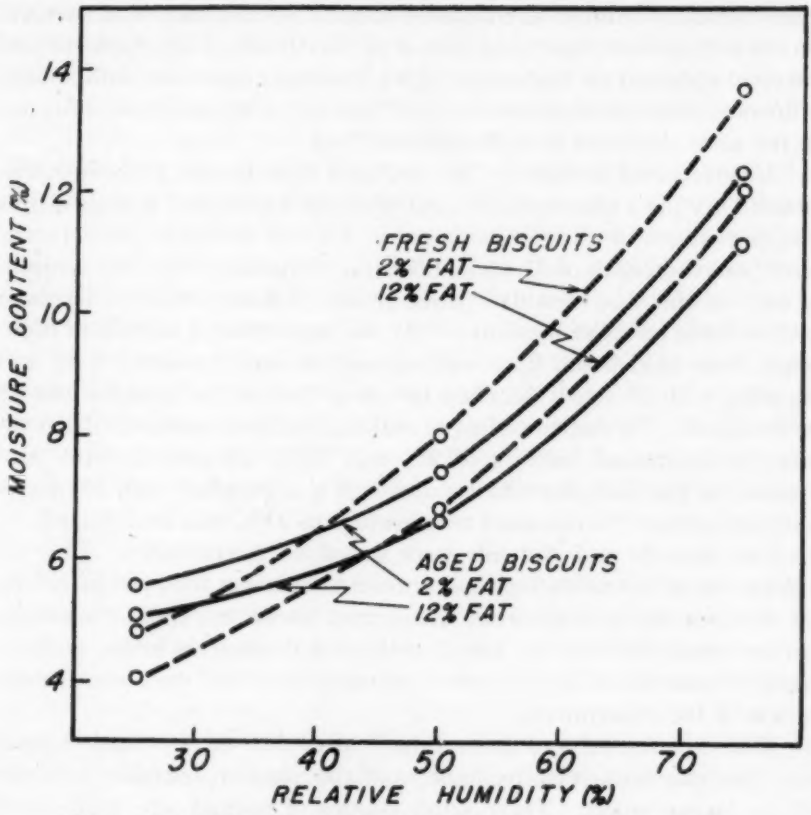

Fig. 3. The hydration capacity of fresh biscuits and biscuits aged for six months.

stored foodstuffs may achieve large numbers and establish a general infestation only when the foodstuff is favorable for their development and survival. This section of the paper deals with the ability of a common stored products insect to develop and survive on biscuits of the same type, and stored under the same conditions, as those described in the preceding section.

The details of the development of certain common insects on raw flour are known, but there is no similar information for insects on

processed flour in the form of biscuits, crackers, etc. The first object of these experiments, therefore, is to compare the development and survival of insects on flour and on biscuits. The second object is to assess the effect on insects of varying the moisture content, flour extraction, and fat level of the biscuits. High moisture content and long-extraction flours are known to favor insect development, but the effects of baking the flour and of fat content are largely unknown. The results indicate that the development and survival of insects on biscuits is greatly reduced as compared to their development and survival on the normal food, flour; and that at the low levels of development and survival obtained on biscuits, a high fat content exerts an unfavorable influence, while moisture content and flour extraction exert an influence in the same direction as with unbaked flour.

Materials and Methods. The confused flour beetle, *Tribolium confusum* Duv., is a cosmopolitan pest of cereal foods, and was chosen as the test insect in these experiments. Fifteen newly-hatched larvae were placed on each of 81 whole biscuits, comprising triplicate samples of each of the nine formulae (three grades of flour and three levels of fat) at three levels of humidity. At the same time, a sample of high-grade household bread flour was exposed to each humidity level and supplied with 10 newly-hatched larvae in two of the samples and 15 in the third. To test the effect of making the food more readily available, finely-ground biscuits of 2% and 12% fat content were also exposed to the three humidities, and each was supplied with 15 newly-hatched larvae. A constant temperature of 27°C was maintained.

The biscuits and controls were examined periodically. The development of larvae on flour and ground biscuit was followed by sifting off the flour and spreading out the ground biscuit to expose the larvae. On the whole biscuits, the larvae concealed themselves in the perforations or penetrated to the inside, so that very few were seen in the course of the experiment.

The experiment was discontinued after 113 days. Each biscuit was carefully broken up by hand, and the number, location, and size of the larvae noted. Three adult beetles of normal size were found inside the biscuits.

Since less than 1% of the original larvae achieved maturity on the biscuits, it was necessary to devise a measure of relative development other than the usual one of the time required to reach maturity. Since adults are actually shorter and weigh less than mature larvae, it is impossible to construct a continuous numerical scale for both larvae and adults in the order of their development.

In order to assess the central tendency in a group containing both adults and larvae at various stages of development, a scale of develop-

ment was devised based on larval size and the achievement of maturity. The surviving larvae were assigned to six size-classes on the basis of their length, according to the measurements of Brindley (1930) for six larval instars. The size-classes were arranged in order of increasing size and successive classes were progressively weighted by unity over the preceding class. This gave a scale of arbitrary values from 1 to 6 on the basis of larval size; adults were assigned a value of 7. The mean of the frequency distribution for larvae and adults according to their values on this scale of development is referred to as the "mean index of development" and provides a method for comparing relative development in groups containing both adults and larvae of various sizes.

The "mean index of development" cannot be interpreted in terms of larval instars or growth stadia. On unfavorable food, such as the biscuits, *Tribolium* larvae may pass through considerably more than the six instars described by Brindley (1930) so that there may have been several instars in any one of the size-classes used in calculating the mean index of development. But where the number of instars is variable because of unfavorable food (Good, 1933), larval size offers a better criterion for assessing the degree of development than the number of moltings. The "mean index of development" as used in this study refers only to insects that survived the 113 days of the experiment; those that died prior to this time were either shrunken so that measurement was impossible or had been eaten by the surviving insects.

Experimental Results. The effect of feeding on flour and on biscuits is sharply reflected in the development and survival of *Tribolium* larvae. Of 810 larvae placed on whole biscuits only three individuals or 0.4% achieved maturity during 113 days, while *all* larvae on flour achieved maturity in an average period of 48 days. On whole biscuits, 6.7% of the insects survived 113 days and achieved a mean development of 4.2, on the developmental scale of 1 to 7. On flour, 88.6% of the insects survived 113 days; all survivors were adults (developmental value of 7) and those that died were adults. The data are shown in Table I. The biscuits at 25% relative humidity were ex-

TABLE I

DEVELOPMENT AND SURVIVAL OF FIRST-INSTAR LARVAE ON FLOUR, WHOLE AND GROUND BISCUITS AT 27°C

Larvae	Flour [1]	Biscuits [2]	
		Whole	Ground
Original number	35	810	60
Percent surviving	88.6	6.7	5.0
Percent achieving maturity	100	0.4	0.0
Mean index of development	7	4.2	3.7

[1] Data after 48 days.
[2] Data after 113 days.

cluded from these comparisons because their moisture content was lower than that of the flour controls.

On ground biscuits, the values for development and survival are about the same as for larvae on whole biscuits. Accordingly, the observed retardation of development and the high mortality of *Tribolium* larvae cannot be attributed to the inability of the larvae to obtain food from the whole biscuits.

Considering the biscuits alone, the effects of moisture content, flour extraction, and fat content may be discerned by comparisons of the development and survival of the 57 insects that remained after 113 days. The data were grouped successively to show the effect of moisture content over all biscuit formulae, flour extraction over all humidities and all fat levels, and fat level over all humidities and all flours. The data are shown in Figure 4.

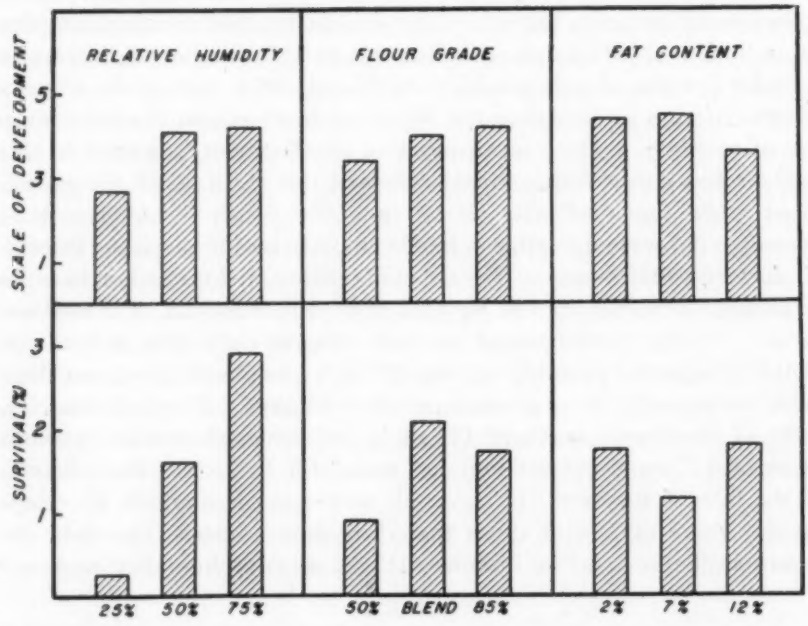

Fig. 4. The mean effect of relative humidity, flour grade, and fat content on the development and survival of larvae reared on biscuits for 113 days at 27°C.

The effect of humidity, or the moisture content of the biscuits (Fig. 4), is to reduce the survival of insects progressively as the relative humidity is lowered from 75% to 25%. The development of larvae is retarded in the same way, very slightly between 75% R.H. and 50% R.H., but abruptly at 25% R.H. On biscuits at 25% R.H. only three larvae survived to achieve a very small growth, at 50% R.H. there was

one adult, and at 75% there were two. The moisture contents of biscuits at 75%, 50%, and 25% relative humidity ranged from 11.8% to 14.1%, 6.5% to 8.1%, and 3.6% to 5.1%, respectively.

The flour grade used in making biscuits influences insect development, long-extraction flour favoring development and refined flour retarding it (Fig. 4). Two larvae reached maturity on biscuits made with a flour of 85% extraction, and one reached maturity on biscuits of 50% extraction flour. Although no larvae reached maturity on biscuits made with a blend of the two flours, there were more in an advanced stage of growth on biscuits made with the blend than on biscuits of 50% extraction flour. Biscuits made with the 50% extraction flour were more unfavorable for insect survival than the other two. Survival appeared to be slightly favored on biscuits made with a blend of the two flours.

The fat content of the biscuits appears to have little effect on the survival of *Tribolium* larvae (Fig. 4). Development however is adversely affected by a fat content of 12%. Biscuits of 7% fat content appear to favor development slightly over biscuits with a fat content of 2%. Two larvae reached maturity on biscuits of 7% fat content and one reached maturity on biscuits of 2% fat; on biscuits of 12% fat there were no adults, and the mean growth of larvae was less than that achieved on biscuits of lower fat content.

The fat content of the biscuits seems to play an important mechanical role in resisting insect attack. Biscuits of 12% fat content are of closer texture and lack the fragile "blisters" that are characteristic of biscuits with a lower fat content. Insect larvae are able to penetrate these blisters and gain access to the inside of the biscuits where they seem to develop more rapidly. The three adult beetles were found *inside* the biscuits, and so was the largest larva. The influence of fat content on larval penetration is shown in Table II.

TABLE II

EFFECT OF FAT CONTENT OF BISCUITS ON PENETRATION OF LARVAE

	2% fat	7% fat	12% fat
Surviving larvae, number	21	15	22
Larvae inside, number	7	6	2
Larvae inside, %	33	40	9

The damage caused by the insects was very slight because so few of them survived to reach an advanced stage of development. The larvae penetrated "blisters," and widened crack-lines on the biscuit surface, often excavating a small cavity in these cracks. Damage of this sort was much more apparent on the biscuits of low fat content

than on those with a high fat content. The cracks and excavations on the low-fat biscuits were increasingly widened and deepened as the experiment continued; but the damage to the high-fat biscuits was scarcely noticeable at the end of the experiment.

Discussion

Biscuits of the type described are unfavorable for the development and survival of *Tribolium* larvae. The results show that, no matter how the moisture content or the principal components of the biscuits are varied, there is a very high mortality of larvae reared on the biscuits, and the development of the few survivors is greatly retarded by comparison with the rate of development on flour. The differences in mortality and development, between larvae reared on flour and those reared on biscuits, are much greater than the differences induced by varying the moisture content and constituents of the biscuits themselves. It is, therefore, reasonable to suppose that merely baking the flour, in the process of biscuit-making, renders it an unsuitable food for the insects. The baking of flour is known to result in loss of thiamine; and thiamine is indicated as a necessary factor for the normal development of *Tribolium* (Street and Palmer, 1935). Moreover, severe losses of thiamine are reported for biscuits baked on the alkaline side (Barackman, 1942), and the basic formula for biscuits used in this study called for 1.5 g of baking soda and no acid salt. Accordingly, it appears that the observed retardation of development and high mortality of *Tribolium* on these biscuits may be related to losses of thiamine during the baking process. This phase of the problem is now in the course of investigation.

The moisture content of the food is an important factor for insect pests of stored products. Both the survival and development of *Tribolium* larvae are reduced as the moisture content of the biscuits is lowered. However, the moisture content of the food is of much less importance than the food itself. For example, all larvae reared on flour of 8.8% moisture content (25% R.H.) completed development, while on biscuits of comparable moisture content (7%–8%) only one of 405 larvae reached maturity. Obviously, the most important factor for *Tribolium* feeding on the biscuits is the nutritional deficiency induced by baking. Superimposed on this major factor is the usual retarding effect when the moisture content of the food is reduced.

The reduction of moisture content obtained by increasing the percentage of fat in the biscuits has no very great effect on *Tribolium*, since the insect has a wide tolerance in moisture requirement, and the reduction of moisture content is small, even for relatively large additions of fat. All larvae achieved maturity on flour within the range

of 14.2% to 8.8% moisture content; and the greatest reduction in the moisture content of the biscuits, obtained by a 10% increase in fat level, was 1.7%. This reduction in moisture content would be chiefly effective against insects on biscuits stored in dry atmospheres.

The observation that at relatively high humidities the hydration capacity of the biscuits decreases with aging indicates that biscuits of this type will become somewhat more resistant to insect attack during storage. The reduction of hydration capacity with age is to be expected since loss of imbibition is a characteristic of staling (Steller and Bailey, 1938); but this does not explain the increase in the hydration capacity of aged biscuits at 25% R.H.

The favorable effect of long-extraction flours over refined flours has been demonstrated for a number of different cereal pests, including *Tribolium* (Good, 1933; Park, 1934; Thomas and Shepard, 1940). The basis for this effect is the absence, in refined flours, of sufficient quantities of minerals and B-complex vitamins. Many of the B vitamins are essential for the normal development of insects that infest stored products (Fraenkel and Blewett, 1943). When flour is baked to produce biscuits, the favorable effect of long-extraction flour over refined flour is still apparent, although at a much lower level than for unprocessed flour. As already suggested, thiamine is the substance that is probably affected by baking. Apparently, the destruction of thiamine during baking is parallel for both long-extraction and short-extraction flours. Biscuits made with a long-extraction flour are therefore more favorable for insects than those made with a short-extraction flour because the thiamine content of the former is higher initially.

The fat content of the biscuits appears to retard development but has no effect on survival. Sweetman and Palmer (1928) found that on an otherwise adequate diet the addition of 10% butterfat or crisco actually accelerated the growth of *Tribolium*. Thomas and Shepard (1940) found that ground walnuts of high oil content were fatal to larvae of the saw-toothed grain beetle, but chopped walnuts had no detrimental effect. In the present study there are no indications that high-fat biscuits, whether whole or ground, are more unfavorable to survival than biscuits of lower fat content.

Summary

Hard biscuits of the type issued to the armed forces, made from three grades of flour and three levels of fat, were stored at three levels of relative humidity to determine the moisture equilibrium values. Increasing the fat content of the biscuits lowered both the initial and the equilibrium moisture content at all levels of relative humidity. Flour grade exerted less influence on moisture content than did fat

content. Aging the biscuits decreased their hydration capacity at high relative humidity and increased it at low relative humidity.

Larvae of the confused flour beetle, *Tribolium confusum* Duv., were reared on the biscuits at three levels of relative humidity. Both the rate of development and the percentage survival of *Tribolium* larvae reared on biscuits were adversely affected as compared to flour controls. Development was greatly retarded and mortality was high for larvae reared on both whole and ground biscuits. It is suggested that destruction of thiamine during the baking process is responsible for the high resistance of the biscuits to insect attack. At the low level of survival and development attained by *Tribolium* larvae on biscuits, high moisture content and biscuits made with a long-extraction flour favored the insects, while biscuits of high fat content were somewhat unfavorable. A high fat content rendered the biscuits less susceptible to penetration by *Tribolium* larvae.

Acknowledgments

The authors wish to acknowledge the assistance of Mr. M. H. Fisher, experimental baker, Grain Research Laboratory, who made the biscuits, and Mr. B. Berck, laboratory assistant, who assisted with all phases of the work.

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THE RELATIVE GERMICIDAL EFFECTIVENESS OF CERTAIN CHLORINE COMPOUNDS ON THE THERMODURIC BACTERIAL SPORES OF WHEAT

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The causative organism of ropiness in baked wheaten products has been variously identified as *B. mesentericus pani viscosus I and II* (Vogel, 1897), *B. mesentericus vulgatus* (Russell, 1898), *B. mesentericus fuscus* (Watkins, 1906), *B. panis* (Fuhrmann, 1905), and *B. mesentericus* (Kent-Jones and Amos, 1930). In any event, the one or more organisms belong to the *B. subtilis* group comprising many widely distributed soil-inhabiting sporulating thermoduric bacilli. It is fairly well agreed they contaminate all wheat, and in the normal milling process they are partly carried into the flour, remaining dormant there, surviving the baking process and exhibiting their presence in the loaf the first or second day following the bake when the interior of the loaf partially liquefies and a foul odor develops.

Since the growth of the bacilli is inhibited by slightly increasing the acidity above that normally encountered in bread, vinegar has long been used as an ingredient of bread during the summer when infection is most likely to appear. Recently, sodium and calcium propionates [1] and sodium diacetate [2] have been utilized for their claimed superior bacteriostatic action.

More basic is the treatment applied within the last few years at the mills. Solutions of chlorine-containing compounds are now used in treating the wheat prior to milling, resulting in a great reduction or actual elimination of rope and mold spores on the wheat. In treating the wheat, advantage is taken of the universal practice of adding 3–4% water to improve the milling qualities. Into the stream of water being blended into the wheat sufficient germicidal solution is fed to provide an available chlorine content of 50–100 ppm, based on the water added. The wheat after being tempered is held in bins for 18–24 hr so that adequate time is allowed for the completion of bactericidal action if sufficient active agent remains present. Because the chloramines are generally more resistant to decomposition in the presence of organic matter, it seemed desirable to determine their relative value in such an application as this.

The object of this work was to ascertain on a laboratory basis the

[1] (Mycobans) E. I. duPont de Nemours and Company, Inc., Wilmington, Del.
[2] Niacet Chemicals Corporation, Niagara Falls, N. Y.

relative germicidal efficiency of Chloramines B and T and a commercial hypochlorite product.

Materials and Methods

The composition of the hypochlorite on analysis of an open market sample was calculated as equivalent to Ca $(OCl)_2$, 17.3%; Ca $(OH)_2$, 9.0%; $CaCl_2$, 6.3%; Na_2CO_3, 66.5%; undetermined, 0.9%. On dissolving in water, sodium hypochlorite is liberated in solution by the reaction

$$Ca(OCl)_2 + Na_2CO_3 = CaCO_3 \text{ (precipitated)} + 2NaOCl.$$

The available chlorine content of the hypochlorite tested was 17.2%. The Chloramines B and T used were of U.S.P. or equivalent grade.

As all wheats, regardless of source, carry a population of the spores under consideration, wheat samples chosen at random may be treated with dilute solutions of germicide and the reduction in population estimated by means of a method such as that of Kent-Jones and Amos (1930). This method is much used by cereal workers, but is rather inaccurate both because of its nature and because of the small number of spores naturally present in normal wheat. First quality cleaned wheat will bear only from 1 to 25 and rarely as high as 50 spores per g, significant commercially, but very low figures for bacteriological test purposes. The test depends on the determination of the smallest amount of wheat which gives in the average case a positive test for presence of spores. When germicidal action is nearly complete, objectionably large portions of ground wheat must be added to the test medium in order to obtain a positive result. Owing to the chance distribution of spores and the other limitations of the method, it is obvious that great variability in results may be expected, and that has been our experience.

By sacrificing some of the *in vivo* aspects of the Kent-Jones and Amos test and substituting more conventional bacteriological methods, a greater degree of precision may be obtained. Pure cultures of the organism were used in the following work and details of their preparation and the test methods used follow.

Several samples of wheats were cultured in nutrient broth, boiled 30 min to destroy all but the thermoduric spores, and incubated. From tubes showing typical pellicles, transfers were made to nutrient agar plates. The best colonies were allowed to grow until the drying of the agar and the overcrowding of the colony were deemed to have encouraged the formation of large numbers of spore forms. Surface scrapings of each selected colony were separately mixed by mortar and pestle with a diluent of sterile silica flour, 250–325 mesh, and heated at 100°C for 20 min to destroy any vegetative forms and any foreign

bacteria possibly included during the operation. The actual concentrations of spores in these silica dilutions were not obtained, but were very high; 5 mg of the material added to 25 ml of liquid insured positive inoculation of culture tubes when only 2 drops of this liquid dilution were used.

Two pure varieties of thermoduric, spore-forming organisms thus were obtained and used as test organisms for the following work. Their cultures on agar plates, in nutrient broth, on potato slants, and microscopic examination indicated that they were strains of *B. subtilis* and *B. mesentericus*. It was demonstrated that the organisms isolated were capable of causing ropiness in baked wheaten products.

Tubes were set up at room temperatures approximating 30°C containing 25 ml of the desired concentrations of germicides, and to each was added approximately 5 mg of the silica flour mixture of the spores. At definite intervals, 2 drops of the solution were added to tubes of nutrient broth, which were immersed in boiling water for 25 min, cooled, and incubated at 30°C for 48 hr. Tubes then showing formation of a pellicle were reported as positive.

Results

In Tables I and II are given the data on the action of various germicides, without the addition of sterile wheat flour in Table I, and

TABLE I

EVALUATION OF BACTERICIDES ON THE SPORES OF B. SUBTILIS AND B. MESENTERICUS

Concentration of available chlorine in ppm	Germicidal agent					
	Chloramine B		Chloramine T		Sodium hypochlorite	
	200 sec	500 sec	200 sec	500 sec	200 sec	500 sec
	B. SUBTILIS					
0	+	+	+	+	+	+
25	+	+	+	+	+	+
50	+	+	+	+	+	−
75	+	−	+	−	+	−
100	−	−	−	−	−	−
150	−	−	−	−	−	−
	B. MESENTERICUS					
0	+	+	+	+	+	+
25	+	+	+	+	+	+
50	+	−	+	+	+	+
75	−	−	+	−	+	+
100	−	−	−	−	−	−
150	−	−	−	−	−	−

TABLE II

EVALUATION OF BACTERICIDES ON THE SPORES OF B. SUBTILIS AND B. MESENTERICUS IN THE PRESENCE OF 50 PPM WHEAT FLOUR

Concentration of available chlorine in ppm	Germicidal agent					
	Chloramine B		Chloramine T		Sodium hypochlorite	
	200 sec	500 sec	200 sec	500 sec	200 sec	500 sec
B. SUBTILIS						
0	+	+	+	+	+	+
25	+	+	+	+	+	+
50	+	+	+	+	+	+
75	+	−	+	+	−	+
100	+	−	−	−	−	−
150	−	−	−	−	−	−
B. MESENTERICUS						
0	+	+	+	+	+	+
25	+	+	+	+	+	+
50	+	+	+	+	+	−
75	+	−	+	+	+	−
100	−	−	−	−	+	−
150	−	−	−	−	−	−

with its addition in Table II, to provide some added organic matter in the system similar to that which in commercial practice is always present.

No differences in resistance to germicidal action beyond those of experimental error of the method seem to exist between the test organisms. The addition of small amounts of wheat flour has shown only the most minor effect though it is possible that quantities of greater magnitude might exert some unfavorable effect.

Finally, the three chlorine germicides, Chloramine B, Chloramine T, and sodium hypochlorite, have averaged almost precisely equal in efficiency when used at the same available chlorine level.

Seventy-five ppm available chlorine is about the critical concentration for 500 sec exposure and 100 ppm must be used for assurance of complete killing. One hundred ppm available chlorine is about the critical concentration for 200 sec exposure and 150 or possibly 125 ppm is required for assurance of complete killing within this shorter period.

Summary

Three germicides have been evaluated for their action on the spores of thermoduric bacteria of wheat, tests being made on the spores in pure cultures.

Sodium hypochlorite, Chloramine B, and Chloramine T compared at equal levels of available chlorine are approximately equally effective towards the organisms tested.

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SOME FACTORS AFFECTING THE DETERMINATION OF RIBOFLAVIN BY THE FLUOROMETRIC METHOD[1]

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Fluorometric methods for the assay of riboflavin have come into wide use for production control purposes as they are more rapid and convenient than the microbiological procedure. The collaborative studies of the Subcommittee on Riboflavin Assay of the American Association of Cereal Chemists (Andrews, 1943, 1943a) have demonstrated that while these two types of assays yield comparable average values, wide variations occur in the results of individual collaborators for each of the methods. Andrews (1943b) has shown that the assay values by both procedures decrease as the quantity of sample taken for analysis is increased. The second collaborative study (Andrews, 1943a) indicated that variations in the efficiency of different lots of "Florisil" to adsorb riboflavin might be a possible source of error in the fluorometric procedure investigated by the subcommittee and it was recommended that the efficiency of Florisil should be checked by adding riboflavin to cereal extracts under study and determining the amount which can be "recovered" by assay. Oxidation with permanganate to destroy interfering pigments was found to be essential in

[1] Paper No. 2199, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis to be presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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the assay of bread but from preliminary experiments it appeared to be unnecessary for enriched flour.

These collaborative studies have shown that the assay of riboflavin is not a simple problem and that there is a need for a detailed investigation of the various steps in the fluorometric procedure. During the course of experiments conducted with the object of developing a rapid fluorometric technique for routine mill control work, some studies were made of the effect of size of sample, of the efficiency of adsorption on Florisil, and of the choice of light filters on assay values. These are presented in this paper.

Experimental

The Reference Method. The assay procedure employed as a standard of reference in these experiments was based principally on the work of the Subcommittee on Riboflavin Assay (Andrews, 1943 and 1943a) with some changes suggested by our own experience. The essential features of the reference method are as follows:

> Suspend 1 g of sample in 50 ml of 0.1*N* H_2SO_4 and digest for 1 hr at the temperature of boiling water. Cool, add 12 ml of 6.5% $Na_2PO_4 \cdot 12H_2O$ and 2 ml of glacial CH_3COOH. Make up to 100 ml with water, add 1 g of Celite and filter. To a 50-ml aliquot of the clear extract add 1 ml of 4% $KMnO_4$ solution, followed, 1 min later, by 1 ml of 3% H_2O_2 solution. The adsorption, elution, and measurement of the riboflavin are carried out as described by Andrews (1943a).

Effect of Sample Size and Florisil Efficiency. Extracts were prepared from 1, 3, 6, and 10 g of second clear flour (Sample No. 1, ash content 1.2%) as outlined in the reference method. Fifty ml of each filtered extract was passed in succession through a series of four adsorption tubes containing 1.1 g of Florisil (Lot No. 1) and the riboflavin adsorbed by each tube was determined. In a parallel experiment, different volumes of one extract (10 g of flour to 100 ml) were each passed through a series of four adsorption tubes and the riboflavin which was taken up in each tube determined. These experiments were repeated later with a new lot of Florisil (No. 2) and another sample of clear flour (No. 2).

The results for equal volumes of clarified extracts representing various weights of flour are recorded in Table I. As the sample size is increased, a smaller fraction of the total riboflavin of the extract is adsorbed in the first tube. Florisil No. 1 required three tubes to adsorb all the riboflavin when a 1-g sample was used, and a single tube adsorbed less than 50% of the riboflavin of the extracts prepared from 6 and 10 g of clear flour. Florisil No. 2 was much more efficient. Despite the fact that adsorption was apparently complete with this Florisil for all the extracts after they had been passed through the third tube, the total recovery decreased with increasing sample size

TABLE I

EFFECT OF SAMPLE WEIGHT ON ADSORPTION OF RIBOFLAVIN FROM CLARIFIED FLOUR EXTRACTS BY FLORISIL

Sample weight	Flour[1] equivalent in aliquot	Riboflavin adsorbed				
		Tube 1	Tube 2	Tube 3	Tube 4	Total
g	g	μg/g	μg/g	μg/g	μg/g	μg/g
FLORISIL NO. 1						
1	0.5	0.95	0.11	0.05	0.00	1.11
3	1.5	0.57	0.24	0.12	0.03	0.96
6	3.0	0.45	0.26	0.16	0.07	0.94
10	5.0	0.44	0.24	0.14	0.09	0.91
FLORISIL NO. 2						
1	0.5	1.15	0.00	0.00	0.00	1.15
3	1.5	0.97	0.08	0.00	0.01	1.06
6	3.0	0.84	0.11	0.03	0.01	0.99
10	5.0	0.76	0.16	0.03	0.01	0.96

[1] The flours employed in preparing the extracts for use with Florisil No. 1 and No. 2 were different samples of clear flour.

so that some factor in addition to Florisil efficiency is apparently partly responsible for the sample size effect. Actually only about one half the lowering of the assay values, obtained by the use of single tubes, which result from taking larger sample weights can be accounted for by incomplete adsorption of the extracted riboflavin by Florisil No. 2. For example, the assay value for the 3-g sample is 0.18 μg/g lower than that for the 1-g sample, and after complete adsorption a difference of 0.09 μg/g still remains; similarly, the assay values for the 5- and 10-g samples, based on the results of single adsorptions, are 0.31 and 0.39 μg/g lower than for the 1-g sample, while the results after complete adsorption are 0.15 and 0.20 μg/g lower.

The results obtained when different volumes of the same clarified extract were each passed through a series of four Florisil tubes are recorded in Table II. Here the differences are due entirely to inefficient adsorption; as the aliquot was increased, more and more riboflavin escaped adsorption by the first tube. Dilution of the aliquots before adsorption had no effect on the results. If, however, 10 g of flour was digested with 50 ml of sulfuric acid and appropriate amounts of sodium phosphate, acetic acid, sulfuric acid, and water added to bring the volume to 1 liter instead of 100 ml and an aliquot oxidized, the same riboflavin values were obtained as when 1 g of sample was digested and diluted to 100 ml.

TABLE II

EFFECT OF ALIQUOT VOLUME ON ADSORPTION OF RIBOFLAVIN FROM CLARIFIED FLOUR EXTRACTS[1] BY FLORISIL

Aliquot	Riboflavin adsorbed				
	Tube 1	Tube 2	Tube 3	Tube 4	Total
ml	*μg/g*	*μg/g*	*μg/g*	*μg/g*	*μg/g*
FLORISIL NO. 1					
25	0.67	0.28	0.07	0.04	1.06
50	0.49	0.32	0.11	0.07	0.99
75	0.34	0.31	0.15	0.14	0.94
100	0.29	0.28	0.14	0.16	0.87
FLORISIL NO. 2					
5	0.92	0.03	0.00	0.00	0.95
15	0.92	0.05	0.01	0.00	0.98
30	0.83	0.13	0.02	0.00	0.98
40	0.76	0.13	0.05	0.01	0.95
50	0.76	0.16	0.03	0.01	0.96

[1] The flours employed in preparing the extracts for use with Florisil No. 1 and No. 2 were different samples of clear flour.

The results given in Tables I and II show that increasing the size of sample has two effects: first it decreases the efficiency of extraction and secondly it decreases the adsorption efficiency of Florisil. With Florisil No. 2, these effects were of approximately equal magnitude. A number of facts lead to the suggestion that soluble substances decrease the adsorption efficiency of Florisil in proportion to the quantities which are present in the clarified extracts. Even Florisil No. 1, which was of low efficiency, adsorbed riboflavin quantitatively from aqueous or buffered acid solutions as a sharp band near the top of the column. The lowered efficiency with flour extracts as the sample size is increased must therefore be ascribed to the presence of increasing quantities of impurities in the extract. That the amount rather than the concentration of these substances is the controlling factor is supported by the fact that dilution of extracts after clarification had no effect on the efficiency of adsorption.

To determine whether pure riboflavin added to a flour extract was adsorbed more readily than that naturally present, an extract of second clear flour (No. 2) was prepared using a sample extractant ratio of 7 g to 100 ml and divided into two equal portions after filtration. To one, pure riboflavin was added in the proportion of 1 μg/10 ml. The enriched and unenriched extracts were each passed through a Florisil tube (Florisil No. 2) and were collected in successive 10-ml

TABLE III

THE FLUORESCENCE OF SUCCESSIVE PORTIONS OF AN EXTRACT OF SECOND CLEAR FLOUR, WITH AND WITHOUT ADDED RIBOFLAVIN, AFTER PASSING THROUGH FLORISIL

Portion	Total of portions	Fluorescence [1]		Riboflavin adsorbed [2]	
		Without added riboflavin	With added riboflavin	Native riboflavin	Added riboflavin
	ml			%	%
1	10	0.0	0.5	100	100
2	20	1.0	1.0	97.5	100
3	30	1.5	1.0	95.8	100
4	40	1.5	1.0	95.0	100
5	50	1.5	1.5	94.5	100
6	60	3.0	3.0	93.0	100
7	70	4.5	5.0	90.7	100
8	80	6.5	6.5	87.8	100
9	90	8.5	10.5	84.4	99.2
10	100	10.0	14.0	81.0	97.9
11	110	14.0	21.5	76.4	95.8
Original Extract		20.0	49.0		

[1] Units of deflection of Coleman photofluorometer less blank.
[2] As percentages of the total quantities passed through the columns, calculated as follows:

$$\% \text{ Native riboflavin adsorbed from 110 ml} = \frac{100 \times [(11 \times 20) - a]}{220} = 76.4$$

$$\% \text{ Added riboflavin adsorbed from 110 ml} = \frac{100 \times [(11 \times (49 - 20))] - (b - a)}{11 \times (49 - 20)} = 95.8$$

Where a equals the sum of the values in column 3, and b the sum of the values in column 4.

portions, the fluorescence of which was measured with the results shown in Table III.

Added riboflavin was completely adsorbed until 80 ml of the extract (containing 8 μg of added riboflavin) had passed through the column. Of the 5.5 μg of native riboflavin in this volume of extract, only 87.8% was adsorbed. It is thus evident that pure riboflavin added to a flour extract may be quantitatively adsorbed, while the riboflavin native to the extract is only partially adsorbed. When more than 80 ml of the solutions were used, it was found that the enriched extract contained more riboflavin after adsorption than the unenriched extract from the other tube, indicating that some of the added riboflavin was leaking through the Florisil. Other experiments showed that the recovery of riboflavin added to filtered second clear extracts decreased as the concentration of the extract increased.

When riboflavin was added to the mixture of flour and extractant either before digestion, or after digestion and cooling, the recoveries were lower than when the addition was made to the clarified extracts. The low recoveries which may be obtained when varying amounts of riboflavin are added to 10 g of second clear flour, either before extraction, or to the flour and extract before dilution, will be seen from the results in Table IV. While the extract was concentrated, only 10 ml

was passed through a Florisil tube in each case. Under these conditions all the added riboflavin, and all except about 0.04 μg of the native riboflavin actually in solution, is adsorbed on the Florisil. Sample No. 2 was used in this experiment and, as shown in Table I, this flour gave a result of 1.15 μg of riboflavin per gram when 1 g was taken for assay.

TABLE IV

RECOVERIES OF RIBOFLAVIN ADDED TO 10 G OF FLOUR BEFORE AND AFTER DIGESTION

Added	Found	Recovery	Riboflavin total Native[1] +added	Total in extract	Total adsorbed on flour
μg/g	μg/g	%	μg	μg	μg
RIBOFLAVIN ADDED BEFORE EXTRACTION					
0.0	0.96	—	11.5	9.6	1.9
1.0	1.80	84.0	21.5	18.0	3.5
2.0	2.69	86.5	31.5	26.9	4.6
3.0	3.47	83.7	41.5	34.7	6.8
RIBOFLAVIN ADDED AFTER EXTRACTION					
0.0	0.93	—	11.5	9.3	2.2
1.0	1.87	94.0	21.5	18.7	2.8
2.0	2.75	91.0	31.5	27.5	4.0
3.0	3.60	89.0	41.5	36.0	5.5

[1] The flour was found to contain 1.15 μg of riboflavin when a 1-g sample was taken for assay.

The low recoveries of added riboflavin were due to the operation of the "extraction factor" which appears from Table IV to be nothing more than the adsorption of native or added riboflavin by the flour residues which are left on the filter paper. The riboflavin thus adsorbed can be released by dilution.

Filters. After testing various Corning glass filters, Conner and Straub (1941) recommended the use of filter 511 for incident light and filter 351 for fluorescent light. They found that an aqueous solution of riboflavin gave the highest fluorescence readings and the lowest blank with this combination of filters. In our assays it proved unsatisfactory. Pyridine eluates are extremely sensitive to ultraviolet light, and their fluorescence dropped very rapidly when filter 511 was used alone for the incident light. On opening the shutter, the galvanometer needle swung over to a maximum and then immediately began to fall off. It seemed certain that all our readings were slightly low and, what was more important, that the error depended upon the variable time required to get a reading after opening the shutter.

A more serious difficulty was that of determining the blank for unenriched patent flours. Each addition of sodium hydrosulfite, after the first one, caused a small decrease in the fluorescence so that it was impossible to select any particular reading as the blank. Eventually both difficulties were overcome by using Corning filter 038 in combination with 511 for the incident light as recommended by Andrews (1943). By means of a Coleman spectrophotometer, set for a wave length of 445 mμ, it was found that filter 038 transmitted only 50% of the incident light. When screened from the light source by filter 038, the fluorescence of pyridine eluates decreased far less rapidly, while the uncertainty as to the blank readings for flour eluates also disappeared.

Since others have experienced trouble in getting satisfactory blank values (Andrews, 1943 and 1943a), the results of an experiment to show the effect of using filter 038 are reported. Additions of a 5% solution of sodium hydrosulfite in ice-cold water were made to 10 ml of a pyridine eluate from flour, and the instrument reading, without filter 038, was taken after each addition. The filter was then inserted and the experiment repeated with another portion of the same eluate. An eluate from a sample of enriched bread was treated in the same manner. The results are shown in Table V.

TABLE V

EFFECT OF ADDITIONS OF SODIUM HYDROSULFITE ON THE FLUORESCENCE READINGS OF PYRIDINE ELUATES WITHOUT AND WITH FILTER 038

Hydrosulfite added	Fluorescence readings [1]			
	Flour		Bread	
	Filter 038		Filter 038	
	Out	In	Out	In
mg				
10	16.2	6.1	10.1	4.5
20	12.4	5.5	8.2	4.1
30	10.5	5.3	7.4	3.7
40	9.1	4.8	7.3	3.7
50	8.7	4.5	6.9	3.5
70	6.8	4.0	5.7	3.4
125	5.0	3.5	5.0	3.1
stimated blank	?	4–5	6–7?	3.5–4.0

[1] Units of deflection of Coleman photofluorometer corrected for dilution; ordinarily the instrument is read to the nearest 0.5 units.

The difficulty of selecting the blank reading for the flour eluate without filter 038 will be obvious. When the filter was used, however, the range of uncertainty did not exceed one degree on the instrument

scale. The advantage of using the extra filter for the bread eluate was less pronounced.

Discussion

Though the choice of a low-grade flour and the use of concentrated extracts for some of the experimental work have exaggerated the difficulties, it is evident that there are many sources of error in the fluorometric procedure, as represented by the reference method, for the determination of riboflavin. Some of the most serious errors are a consequence of the limitations of Florisil as an adsorbent for riboflavin. These limitations vary from one lot of Florisil to another [5] and have a greater or lesser effect upon the assay results depending upon the concentration, volume, and clarity of the extracts from which the riboflavin is adsorbed. In a rapid method for the determination of riboflavin described by the authors, the adsorption step has been eliminated [6] (Hoffer, Alcock, and Geddes, 1944).

Since added riboflavin was more readily adsorbed by Florisil than the riboflavin native to flour extracts, good results of recovery tests can never justify confidence in the results of riboflavin assays. This does not mean that such tests are valueless, for low recoveries will certainly indicate low assay values. The test should be carried out, however, by adding the riboflavin before digestion and using the same weight of sample and the same aliquot volume as are used in the assay itself. The galvanometer reading must then be brought within the desired range by taking a suitable volume of eluate. The quality of a new lot of Florisil can best be checked by comparing it with satisfactory material. When this cannot be done, useful information as to the limitations of the Florisil may be secured by passing extracts through a number of adsorption tubes in series as described earlier.

Other practical consequences of these studies were that in later work no more than 1 g of sample was taken for assay, and, to be on the safe side, the amount of Florisil used per tube was increased from 1.1 to 1.8 g (6.25 cm).

Our experience also indicates the need for carrying out the adsorption from perfectly clear extracts. We have found a No. 40 Whatman paper suitable for filtering flour extracts and that filtration can be speeded up by use of Celite. This material does not adsorb riboflavin.

Pure riboflavin adsorbed from aqueous solution is very firmly held by Florisil and cannot be removed by washing with a large volume of

[5] The Floridin Company informed us that these variations have now been eliminated.

[6] While our studies were in progress, adsorption on Florisil was one of the steps in the fluorometric analyses of enriched flour and bread which was under investigation by the 1943–44 Subcommittee on Riboflavin Assay. The report of their studies (Cereal Chem. 21: 398–407, 1944) has appeared since the submission of this manuscript and confirms our finding that the adsorption step can be omitted.

water. Native riboflavin adsorbed from flour extracts, and possibly from other materials, is much less firmly held and some losses may occur when the Florisil is washed. Accordingly, the minimum amount of water (not more than 10 ml) should be used for washing.

Summary

A number of sources of error in the fluorometric method for the determination of riboflavin have been studied.

Two factors may contribute to the sample size effect. They are: (1) Adsorption of riboflavin on the residues remaining after extraction. (2) Less complete adsorption by Florisil of the riboflavin from more concentrated extracts. The magnitude of the effect of the second factor depends upon the aliquot volume and the quality of the Florisil. Riboflavin adsorbed on the extraction residues goes into solution upon dilution.

Pure riboflavin is adsorbed more readily by Florisil than the native riboflavin of flour extracts. This difference must be taken into account when considering the significance of recovery tests. Suggestions have been made for carrying out recovery tests and for checking the adsorbing qualities of Florisil.

Extracts should be freed from any suspended material before they are passed over Florisil. Native riboflavin adsorbed on Florisil may be removed by washing, and accordingly the minimum amount of wash water should be used.

The use of Corning filter 511 alone for the incident light proved unsatisfactory owing to the photosensitivity of riboflavin in pyridine eluates and to the difficulty of finding the blank values for patent flour eluates. The use of a combination of Corning filters 511 and 038 for the incident light with 351 for the fluorescent light eliminated these difficulties as sources of important errors.

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A RAPID METHOD FOR THE DETERMINATION OF RIBOFLAVIN IN WHEAT AND WHEAT PRODUCTS [1]

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The need for a rapid precise assay of riboflavin in wheat and wheat products led to an investigation of the possibility of using a portion of the extract obtained in the determination of thiamine by the rapid method described by the authors (Hoffer, Alcock, and Geddes, 1943). From this starting point the rapid method of riboflavin assay described in this paper has been developed and employed in the analysis of wheats, flour and feed streams, and enriched flours in comparison with a reference procedure. The use of Florisil and pyridine is eliminated and a single determination can be made in 60 to 70 min.

The Reference Method

The assay procedure employed as a standard of reference in these experiments was a modification of the fluorometric method used by the Subcommittee on Riboflavin Assay (Andrews, 1943, 1943a). The essential features of this reference method have been described by Hoffer, Alcock, and Geddes (1944) in connection with their study of certain steps in the fluorometric method. In view of the results of the experiments on light filters, all the reference method values given in the present paper were obtained by using a combination of Corning glass filters 511 and 038 for the incident light and 351 for fluorescent light.

The Rapid Method

After some preliminary studies the following rapid method for the determination of riboflavin in wheat and wheat products was developed.

Apparatus

(1) Photofluorometer.
(2) Centrifuge and 50-ml centrifuge tubes, preferably without lip.
(3) Boiling tubes.

Reagents

(1) Potassium chloride solution. Dissolve 250 g in 2% acetic acid and make up to 1 liter.
(2) Potassium permanganate solution. 0.5% freshly prepared.
(3) Hydrogen peroxide 0.3%.
(4) Sodium fluorescein. Stock solution—10 mg to 1 liter. Working standard (0.05 μg/ml)—dilute stock solution 5 to 1000.
(5) Sodium hydrosulfite.
(6) Riboflavin standard. 1 μg/ml.

[1] Paper No. 2200, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis to be presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Chemist, Western Canada Flour Mills Co. Ltd., Winnipeg, Manitoba.
[3] Chief Chemist, Western Canada Flour Mills Co. Ltd., Winnipeg, Manitoba.
[4] Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

Method. Place 0.5 g of sample in a 50-ml centrifuge tube with 20 ml of the KCl solution. Break up any lumps by means of a glass rod. Place the centrifuge tube in a water bath at 70°C, cover with a boiling tube to prevent evaporation, and leave for 30 min. At the end of this time, stir the mixture to break up any lumps that have formed, and then centrifuge at 2500 rpm for at least 10 min.

Transfer a 5-ml aliquot of the supernatant solution to a cuvette and add 5 ml of water. Now add rapidly 1 ml of $KMnO_4$ solution and, after ½ min, 1 ml of H_2O_2. After the gas bubbles have cleared, read the fluorescence (A). Add 1 ml of riboflavin standard and again read the fluorescence (B). Add about 30 or 40 mg of solid sodium hydrosulfite, followed by further additions of about 10 mg each, and read the fluorescence after each addition. When an addition of hydrosulfite fails to reduce the reading, that reading is taken as the blank fluorescence (C).

The riboflavin content of the sample is calculated as follows:

$$\text{Riboflavin } \mu g/g = \frac{A - 1.08C}{1.08B - A} \times 8.$$

The use of the factor 1.08 is necessitated by the increase in the volume of the liquid in the cuvette from 12 ml to 13 ml upon the addition of the riboflavin standard.

Sample Size Effect in the Rapid Method. That sample size affects assay values obtained by the rapid method is evident from the results in Table I.

TABLE I

EFFECT OF SAMPLE SIZE ON THE RESULTS OF ASSAYS FOR RIBOFLAVIN BY THE RAPID METHOD

Weight of sample	Riboflavin content					
	Patent flour	Enriched flour	Enriched bread	Whole wheat	Bran	Shorts
g	*μg/g*	*μg/g*	*μg/g*	*μg/g*	*μg/g*	*μg/g*
1.50	0.20	2.15	2.70	1.04	2.39	2.47
1.00	0.22	2.10	2.77	1.06	2.57	2.85
0.75	0.26	2.31	2.82	1.07	2.87	2.99
0.50	0.29	2.44	3.09	1.16	2.98	3.17

In order to minimize the effect of sample size, the weight of sample taken for assay by the rapid method was fixed at 0.5 g. It was impracticable to use a smaller quantity because of the low fluorescence readings of the extracts.

Filters. The Corning 351 filters supplied with the Coleman photofluorometer vary considerably in density. With the same solution of sodium fluorescein and the same setting of the diaphragm, three filters gave the following instrument readings: 60, 49, and 40. All the work reported here was done with the least dense of these three filters and even then the readings for unenriched patent flours were very low.

For the purpose of increasing the fluorescence readings, filter 038 was removed when dealing with rapid method extracts. No difficulty was experienced in getting satisfactory blank readings without this filter, as will be apparent from Table II which shows the effect of additions of sodium hydrosulfite on the fluorescence of rapid method extracts.

TABLE II

EFFECT OF ADDITIONS OF SODIUM HYDROSULFITE ON THE FLUORESCENCE READINGS OF RAPID METHOD EXTRACTS—WITHOUT FILTER C. G. 038

Hydrosulfite added	Fluorescence readings [1]		
	Flour	Bread	Bran
mg			
10	1.5	3.5	15.0
20	1.5	2.8	8.6
30	1.3	2.5	6.1
40	1.0	2.4	5.1
50	—	2.3	5.2
60	—	2.3	4.7
70	—	2.5	4.7
80	—	—	4.5
90	—	—	4.3
100	1.0	—	4.3
Estimated blank	1.0	2.5	4.5–5.0

[1] Units of deflection of Coleman photofluorometer corrected for dilution. Ordinarily the instrument is read to the nearest 0.5°.

Rapid method extracts have a lower fluorescence and are much less sensitive to ultraviolet light than pyridine eluates, even though 25% potassium chloride in 2% acetic acid transmits 99% of the light transmitted by water. Because of this greater stability, riboflavin assays by the rapid method can be made in diffused daylight. When using the reference method, the blinds were drawn, the laboratory was illuminated with orange-colored lights, and solutions containing riboflavin were covered with a black cloth.

It should be noted that 2 or 3 min after the first addition of sodium hydrosulfite to a rapid method extract, the solution starts to become opalescent owing to the precipitation of sulfur. There is, however, plenty of time before this occurs to determine the blank. In the case of pyridine eluates, the released sulfur is dissolved, causing the solution to turn yellow.

Concentration of Potassium Chloride for Extraction. Because 25% potassium chloride in 2% acetic acid was used as the extractant in the rapid method for thiamine assay, it was also used for the riboflavin determination. But when it was decided to fix the sample weight at 0.5 g for riboflavin assays, and it became no longer possible to consider the routine use of portions of the same extract for both thiamine and riboflavin determinations, no strong *a priori* reason for using this particular extractant remained.

In the method as outlined above, 5 ml of extract is diluted with 5 ml of water before oxidation with permanganate. Before trying other concentrations of potassium chloride, a few determinations were made

TABLE III

RIBOFLAVIN VALUES AS DETERMINED BY MEASURING THE FLUORESCENCE OF UNDILUTED AND DILUTED RAPID METHOD EXTRACTS AND BY THE REFERENCE METHOD

Sample	Riboflavin content		
	Rapid method		Reference method
	Undiluted extract	Diluted extract	
	μg/g	μg/g	μg/g
Bread	3.44	3.21	3.34
Bran	4.23	3.13	3.23
Shorts	3.76	2.91	2.60
Whole wheat	1.39	1.21	1.21

using 10 ml of the undiluted extract for fluorometric measurement. The results are given in Table III.

The diluted extract gave results closer to those obtained by the reference method than did the undiluted extract. Another disadvantage of the undiluted extract was that it foamed excessively when hydrogen peroxide was added and took a longer time to clear.

Extractions with various concentrations of potassium chloride in 2% acetic acid gave the results reported in Table IV. In each case 5 ml of the extract was diluted with 5 ml of water before the addition of permanganate.

TABLE IV

RIBOFLAVIN VALUES AS DETERMINED BY EXTRACTION WITH VARIOUS CONCENTRATIONS OF POTASSIUM CHLORIDE

KCl concentration	Riboflavin content						Fluorescence reading[1]—1 μg of riboflavin
	Flour	Bread	Bran	Shorts	Whole wheat	Germ	
%	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	
0	—	3.21	2.90	3.20	1.32	4.47	71
5	0.32	3.35	2.64	3.59	1.12	4.48	60
10	0.34	3.13	3.24	3.00	1.13	5.08	52
15	0.34	3.27	3.29	2.80	1.24	5.04	48
20	0.37	3.16	3.24	2.87	1.25	5.06	45
25	0.34	3.21	3.13	2.91	1.21	5.28	42
Reference method	0.32	3.34	3.24	2.60	1.21	5.03	—

[1] Obtained by adding 5 ml of water, 1 ml of $KmnO_4$ solution, 1 ml of H_2O_2 solution, and 1 ml of the riboflavin standard to 5 ml of KCl solution of the indicated concentration.

Concentrations of 0% and 5% were ruled out because they yielded cloudy extracts in the case of flour, shorts, and whole wheat. There was little to choose between the other concentrations of potassium

chloride and, as some data had already been accumulated using the 25% solution, it was employed for the rest of the work.

Application of the Rapid Method to Flours and Mill Feeds

All the flour and feed streams from a large commercial mill were analyzed for riboflavin, in duplicate and on different days, by the rapid

TABLE V

RIBOFLAVIN VALUES ON FLOUR AND FEED STREAMS AS DETERMINED BY THE REFERENCE METHOD AND BY THE RAPID METHOD WITH AND WITHOUT PERMANGANATE TREATMENT

Stream	Ash	Riboflavin content		
		Reference method	Rapid method	
			With $KMnO_4$	Without $KMnO_4$
	%	μg/g	μg/g	μg/g
(a) 2nd middlings	0.34	0.34	0.35	0.36
4th middlings	0.37	0.42	0.33	0.41
1st middlings	0.38	0.34	0.35	0.46
3rd middlings	0.39	0.47	0.36	0.41
Coarse sizer	0.41	0.44	0.31	0.34
Fine sizer	0.43	0.38	0.39	0.47
5th middlings	0.44	0.44	0.36	0.39
6th middlings	0.48	0.41	0.40	0.51
7th middlings	0.49	0.40	0.37	0.56
2nd and 3rd break	0.51	0.49	0.38	0.53
1st tailings	0.54	0.55	0.43	0.61
Roll dust	0.55	0.47	0.46	0.54
8th middlings	0.61	0.55	0.49	0.72
4th break	0.68	0.41	0.43	0.60
2nd quality	0.72	0.59	0.56	0.67
Purifier dust	0.72	0.57	0.49	0.63
1st break	0.72	0.59	0.56	0.70
1st middlings scalp	0.75	0.53	0.55	0.67
1st chip	0.84	0.69	0.67	0.80
1st germ	0.93	0.69	0.66	0.87
1st low grade	0.96	0.79	0.69	0.86
5th break	1.12	0.85	0.81	1.06
2nd germ and chip	1.28	0.99	0.88	1.18
2nd low grade	1.37	1.01	0.95	1.13
Mean—all flour streams		0.56	0.51	0.65
(b) Low grade tailings	1.78	1.06	0.91	1.36
3rd low grade	2.04	1.66	1.60	1.95
Bran and shorts duster redresser	2.25	1.61	1.55	2.23
Bran and shorts reel	2.30	1.68	1.50	2.05
Feed middlings	2.90	2.52	2.62	3.91
Feed middlings	3.31	2.37	2.30	3.38
Feed middlings	3.75	3.34	3.31	4.89
Shorts	4.19	2.99	3.18	5.53
Bran	5.76	2.56	2.65	4.28
Mean—all feed streams		2.20	2.18	3.28
Mean—for all samples		1.01	0.97	1.36

method with and without permanganate treatment. The feed samples were analyzed in duplicate on different days by the reference method, but the reference method values for the flour samples were the results of single determinations. The results are given in Table V.

Generally speaking, there was excellent agreement between the values obtained by the rapid method using permanganate and by the reference method. The results of a statistical analysis of the values in Table V are given in Table VI.

TABLE VI

MEANS, STANDARD DEVIATIONS, AND CORRELATION COEFFICIENTS OF RIBOFLAVIN VALUES AS DETERMINED BY THE REFERENCE METHOD AND THE RAPID METHOD (Using permanganate)[1]

Material	Mean		Standard deviation		Correlation coefficient
	Reference	Rapid	Reference	Rapid	
Flour streams	0.56	0.51	0.19	0.18	0.970
Feed streams	2.20	2.18	0.70	0.78	0.994
All streams	1.01	0.97	0.83	0.86	0.997

[1] Statistical summary of data in Table V.

The results of assays by the rapid method were slightly lower on the average than those obtained by the reference method. Although this difference is statistically significant it is so small that for practical purposes it can be ignored.

The rapid method assays without permanganate were carried out to determine whether oxidation of the extract was essential. It will be seen from Table VII that the effect of permanganate treatment increased as the ash content of the material increased.

For lower grade flours and feeds, treatment of rapid method extracts with permanganate is obviously necessary, but with flours containing up to 0.60% ash, the results were equally good whether permanganate was used or not. For enriched and unenriched patent flours, oxidation with permanganate can therefore be omitted, although this step was carried out in securing the rapid method results still to be considered.

Application of the Rapid Method to Whole Wheat

Sixteen samples of whole wheat, ground so that all except a small amount of branny material passed a 44 GG sieve, were analyzed in duplicate on different days by both methods. The means of the results were as follows:

Reference method	1.12 μg/g
Rapid method	1.08 μg/g

TABLE VII

MEAN RIBOFLAVIN VALUES FOR MILL STREAMS GROUPED ACCORDING TO ASH CONTENT; AS DETERMINED BY THE REFERENCE METHOD AND THE RAPID METHOD WITH AND WITHOUT PERMANGANATE

No. of samples	Ash range	Riboflavin content		
		Reference method	Rapid method	
			With $KMnO_4$	Without $KMnO_4$
	%	μg/g	μg/g	μg/g
4	0.35–0.39	0.39	0.35	0.41
5	0.40–0.49	0.41	0.37	0.45
3	0.50–0.59	0.50	0.44	0.56
6	0.60–0.79	0.54	0.51	0.67
3	0.80–0.99	0.72	0.67	0.84
4	1.00–1.99	0.98	0.89	1.18
4	2.00–2.99	1.87	1.82	2.54
2	3.00–3.99	2.86	2.81	5.21
2	4.00–5.99	2.78	2.92	3.79

As was the case with the mill streams, the rapid method gave results that, on the average, were 0.04 μg/g lower than those yielded by the reference method. Though small this difference was significant.

Precision of the Reference and Rapid Methods

The standard errors and coefficients of variability for different materials are shown in Table VIII.

TABLE VIII

STANDARD ERRORS AND COEFFICIENTS OF VARIABILITY OF THE REFERENCE METHOD AND THE RAPID METHOD

Material	Method	Number of samples	Mean riboflavin content	Standard error [1] Single determination	Coefficient of variability
			μg/g	μg/g	%
Flour [2]	Rapid	24	0.50	0.04	8.0
Mill feed	Reference	9	2.20	0.29	13.1
	Rapid	13	2.36	0.13	5.5
Wheat	Reference	16	1.12	0.07	6.3
	Rapid	16	1.08	0.06	5.6
Wheat kernels glumes and stems [3]	Reference	29	1.54	0.14	8.8
	Reference	56	3.17	0.23	7.1
	Reference	45	4.98	0.36	7.2
	Reference	57	6.94	0.44	6.4
	Reference	9	8.70	0.44	5.0

[1] From results of duplicate determinations on different days.
[2] Duplicate determinations on different days were made by the reference method on these 24 samples of flour using 5 g of sample. The mean riboflavin content was 0.47 μg/g, the standard error of a single determination 0.07 μg/g, and the coefficient of variability 14.9%. Single determinations by the reference method using 1 g samples gave a mean value of 0.56 μg/g.
[3] Samples at different stages of development obtained in connection with another study.

For both methods the standard error in absolute terms increased with the riboflavin content, but on a percentage basis it decreased with increasing riboflavin content. As might be expected in view of its greater simplicity, the error for the rapid method was lower than for the reference method. Although the percentage error for the rapid method may still appear to be large, especially in the case of flours, the absolute error was within what may be regarded as acceptable limits for a determination of this nature.

Riboflavin values for several collaborative check samples of enriched flour and bread have been determined by the reference and rapid methods. The results are given in Table IX together with the mean, minimum, and maximum values for the collaborators. The authors' data on an additional series of wheat flours which had been analyzed in another laboratory are given in Table X. These comparative values

TABLE IX

A Comparison of Riboflavin Values for Check Samples

A.A.C.C. collaborative samples	Riboflavin content					
	Collaborative values		Authors' values			
			Reference method		Rapid method	
	Mean	Range	Mean	Range	Mean	Range
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
April 1943 enriched flour	2.98	2.29–4.00	2.93	2.78–3.07	2.83	2.73–2.99
October 1943 enriched flour	3.15	2.20–4.39	2.75	2.50–2.86	3.01	2.96–3.09
December 1943 enriched flour	2.62	1.48–3.39	3.12	2.86–3.47	2.94	2.90–2.98
February 1944 enriched bread	2.60	1.54–3.44	2.92	2.85–3.00	2.69	2.65–2.84
Mean	2.84		2.93		2.87	

TABLE X

A Comparison of Riboflavin Values for Samples Supplied by J. S. Andrews [1]

Material	Andrews' values		Authors' values	
	Microbiological	Fluorometric	Reference method	Rapid method
Patent flour	0.36	0.32	0.28	0.28
Enriched patent	2.60	2.58	2.49	2.45
Enriched bread	2.66	2.95	3.34	3.03
Whole wheat flour	1.11	1.13	1.14	1.21
Mean	1.68	1.75	1.81	1.74

[1] General Mills Inc., Research Dept., Minneapolis, Minnesota.

show that the authors' results are in good agreement with those obtained by other laboratories.

Discussion

The elimination of adsorption on Florisil, with all the hazards attending this step, is the chief advantage of the rapid method.[5]

The use of the rapid method not only reduces the duplicate error but also results in a considerable saving of time. A single determination by the reference method takes 2½ to 3 hr and by the rapid method not more than 70 min. A patent flour, enriched or unenriched, can be easily assayed in 1 hr, since in this case it is unnecessary to oxidize the extract with permanganate. Another advantage of the rapid method is the avoidance of the use of pyridine.

The low fluorescence of extracts of unenriched patent flours constitutes the chief drawback of the rapid method. A flour containing 0.4 μg/g of riboflavin yields an extract with a fluorescence reading of 3 and a blank of 1 on our Coleman photofluorometer.

Even when the reference method is used for assaying unenriched patent flours, some special difficulties are encountered owing to their low riboflavin content, for, in order to get a satisfactory concentration of riboflavin in the eluates, one must run the risks of error associated with the use of larger samples or larger aliquots. However, on account of their low and comparatively uniform riboflavin content, the analyst is perhaps least interested in unenriched patent flours, though the fact that they can be satisfactorily assayed by the rapid method, in spite of the low fluorescence of their extracts, is made evident by the data which have been presented.

With enriched patents, the rapid method readings are much better. A sample containing 3.0 μg/g gives a reading of approximately 18 with a blank of 2. The rapid method also works well with enriched bread, whole wheat, wheat germ, and mill feeds, while a few tests have indicated that it can be adapted for assaying dry and liquid milk. It is unsuitable for assays on green plant tissue.

Summary

A rapid and relatively simple method for the assay of riboflavin in wheat and wheat products has been described. It eliminates adsorption on Florisil and the use of pyridine. A single determination can be made in 60 to 70 min.

[5] During the course of our studies the effect of Florisil and permanganate in the fluorometric analysis of enriched flour and bread was under investigation by the 1943–44 Subcommittee on Riboflavin Assay. The report of these studies (Cereal Chem. 21: 398–407, 1944), which appeared since the submission of this manuscript, showed that the use of Florisil resulted in poor agreement between laboratories and indicated that relatively simple assay methods could be employed. With enriched flours direct reading of flour extracts gave just as satisfactory a result as when either Florisil or permanganate was employed. In assaying enriched bread, treatment of the extract with Florisil had no effect on the final results but the use of permanganate aided in removing interfering impurities.

Results of assays of flour, mill feeds, and bread by the rapid method agreed closely with those obtained by a modification of the regular fluorometric procedure employed as a standard of reference.

For both the rapid and reference methods, the absolute error increased, while the percentage error decreased, with increasing riboflavin content. The duplicate error for the rapid method was lower than for the regular method.

The rapid method works well with enriched flour and bread, mill feeds, and wheat, and there are indications that it can be applied to other materials, though not to green plant tissues. In spite of low fluorescence readings it is capable of giving satisfactory results for unenriched patent flours.

Acknowledgment

The authors wish to acknowledge their indebtedness to J. S. Andrews, General Mills Inc., Research Department, Minneapolis, Minnesota, for furnishing four samples with information as to their riboflavin content.

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A MODIFICATION OF THE KNEEN AND SANDSTEDT METHODS FOR THE DETERMINATION OF ALPHA- AND BETA-AMYLASES IN BARLEY MALT [1]

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Methods for the determination of alpha- and beta-amylase in malt have been developed by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941). The methods were designed as a measure of alpha-dextrinizing, malt (alpha- + beta-) saccharifying activities, and, by difference, the beta-saccharifying activity of malt. There seemed no objection to adapting the determination of alpha-dextrinizing activ-

[1] Cooperative investigations between the University of Wisconsin and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

ity to 20°C and substituting the regular diastatic procedure for the malt-saccharifying activity determination proposed by these workers (1941). This change would make the method more conveniently applicable in malt laboratories using the diastatic power procedure. This modification has involved the establishment of the relation between saccharification and dextrinization of alpha-amylase under the modified experimental conditions. Another method of preparation of enzymatically pure beta-amylase has been tested and found to be satisfactory in the preparation of the standard alpha-amylodextrin substrate.[2] A new unit is proposed for alpha-amylase based upon the diastatic power unit in maltose equivalent.[3]

Preparation of Amylase Components

It was essential that enzymatically pure preparations of both alpha- and beta-amylase be made. The alpha-amylase preparations were used in the establishment of the relation existing between alpha-saccharifying and alpha-dextrinizing activities, and the beta-amylase in the preparation of the alpha-amylodextrin substrates.

Malt Alpha-Amylase. The method of Ohlsson as modified by Kneen, Sandstedt, and Hollenbeck (1943) was used in the preparation of malt alpha-amylase.

Barley Beta-Amylase. Two methods have been used for the preparation of barley beta-amylase. The first is that described by Kneen, Sandstedt, and Hollenbeck (1943). The second is a modification of the fractional alcohol precipitation technique used by Meyer, Bernfeld, and Press (1940) for wheat. Briefly, the latter method involves: (1) concentration of a 1 : 5 aqueous barley extract to one third its volume under reduced pressure, (2) making the concentrated extract up to an ethanol concentration of 65% in the cold, separating and discarding the material settling out, and (3) raising the ethanol concentration of the clear supernatant to 80% ethanol in the cold, and isolation of the separated material.

Beta-amylase preparations made by the technique of Kneen, Sandstedt, and Hollenbeck (1943) were less soluble in water than those prepared by the fractional alcohol precipitation technique but were equally satisfactory when the test described later in this paper was applied to them. While considerable individual variation was apparent, the saccharifying activities of preparations made by both techniques were of the same order.

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[2] Alpha-amylodextrin is the limit dextrin produced by the action of beta-amylase on soluble starch.

[3] "Maltose equivalent" is the unit of diastatic power preferred to the older "degrees Lintner" by the American Society of Brewing Chemists. Maltose equivalent is defined as "the grams reducing substances expressed as the grams maltose produced from 100 g malt acting on soluble starch under the specified experimental conditions." "Degrees Lintner" is obtained by dividing the maltose equivalent by 4.

Test for Purity of Beta-Amylase

A test for the absence of alpha-amylase in the beta-amylase is necessary when the beta-amylase is to be used in the preparation of the standard amylodextrin substrate. The proposed test is simple in principle, and has been found to be sensitive to small contamination by alpha-amylase. The test also serves to fix the minimal quantity of beta-amylase essential for complete conversion of the starch to amylodextrin in 24 hr.

To 50 ml portions of 4% soluble starch (special for diastatic power) in 100 ml volumetric flasks are added increasing amounts of the beta-amylase preparation dissolved in 5 ml buffer mixture (Sandstedt, Kneen, and Blish; 1939) with or without additional water. Obviously, the range of additions of beta-amylase to the starch is determined by the activity of the preparation tested. A six-fold range in concentration has been found satisfactory. The volumetric flasks are made to the mark with water, toluene added, the contents mixed well, and allowed to stand 24 hr at room temperature.

After 24 hr, dextrinization measurements are made upon the various substrates by the method of Sandstedt, Kneen, and Blish as modified in this paper, using a standard source of malt alpha-amylase as dextrinizing agent. A constant dextrinizing time over a range of beta-amylase concentration is indicative of freedom from alpha-amylase contamination. The use of amylase preparations of bacterial, fungal, or animal origin instead of malt alpha-amylase is not recommended, as the application of the method to these enzymes has not been established. In the preparation of the standard amylodextrin substrate, the lowest concentration at which a constant dextrinizing time is attained in the test above, plus 25% excess, has been used.

Relation between Saccharifying and Dextrinizing Activities of Alpha-Amylase

In order to correct for the saccharification by alpha-amylase in the diastatic power determination, it is essential that the relation between the dextrinizing and saccharifying activities of alpha-amylase be established. By use of this relationship, the saccharification by beta-amylase can be calculated accurately and expressed as the difference between the malt and alpha-amylase saccharification.

The saccharification by various quantities of alpha-amylase was carried out in duplicate by use of the diastatic power procedure (Cereal Laboratory Methods; 4th ed., 1941). Dextrinization measurements of the same quantities of alpha-amylase used in the saccharification measurements were made by the method of Sandstedt, Kneen, and

Blish (1939) as modified herein. These dextrinization values were obtained in duplicate on each of two substrates. The substrates were made with two different beta-amylase concentrates, prepared by each of the two methods mentioned above. Agreement between the four dextrinization determinations was very good, if dextrinization times below 10 min were avoided.

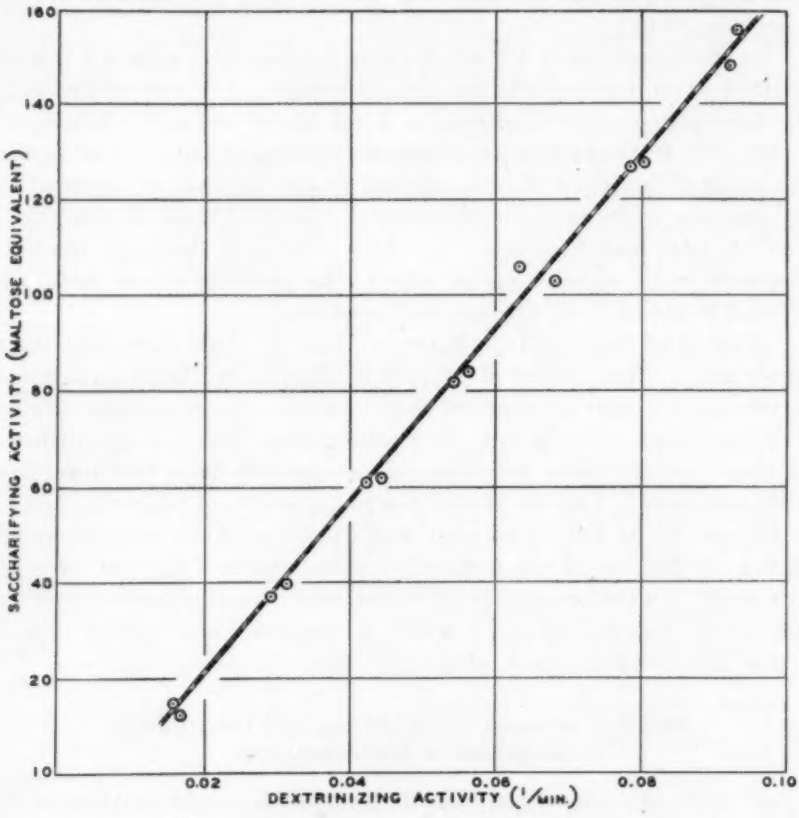

Fig. 1. The relation between alpha-amylase dextrinizing and saccharifying activities.

The saccharification values were calculated by multiplying the ml potassium ferricyanide reduced by 144, the factor used in calculating the diastatic power (maltose equivalent), when 1 ml (0.05 g malt equivalent) diastatic extract is used in the diastasis. The relation between the average saccharification values expressed in maltose equivalent and the dextrinizing activity expressed as reciprocal minutes is shown in Figure 1. A series of values was read from the graph, and is given in Table I.

TABLE I

SACCHARIFICATION EQUIVALENTS "K" (MALTOSE EQUIVALENT) OF DIFFERENT QUANTITIES OF ALPHA-AMYLASE, EXPRESSED IN RECIPROCAL MINUTES

Dextrinization 1/min	Saccharification equivalent "K"	Dextrinization 1/min	Saccharification equivalent "K"
0.020	21	0.065	103
0.025	30	0.070	111
0.030	39	0.075	121
0.035	48	0.080	130
0.040	57	0.085	139
0.045	66	0.090	148
0.050	75	0.095	157
0.055	84	0.100	165
0.060	93		

The Proposed Methods for Alpha- and Beta-Amylase in Malt

The reagents and procedure are the same as given by Sandstedt, Kneen, and Blish (1939) for the determination of alpha-amylase except that (1) 20°C instead of 30°C was used for the reaction, and (2) extraction of the malt was carried out according to the Cereal Laboratory Methods (4th ed., 1941) diastatic power procedure. For the determination of malt-saccharifying activity, the diastatic power procedure (Cereal Laboratory Methods; 4th ed., 1941) is followed exactly as published.

Minor changes in the method of color comparison are being used successfully in the Barley and Malt Laboratory at Madison. An inorganic color standard suggested by Landis [4] was modified slightly and substituted for the dextrin standard described by Sandstedt, Kneen, and Blish. This standard is prepared as follows: 25 g $CoCl_2 \cdot 6H_2O$ and 3.84 g $K_2Cr_2O_7$ per 100 ml 0.01*N* HCl.

Color comparisons were made visually through 6.0–6.2 cm (6 ml) of the iodine-digest complex in 4-inch test tubes set over a 15 watt daylight fluorescent bulb against 6.0 ml of the color standard described above. In using this procedure it is essential that a set of test tubes be obtained varying by not more than 2 mm in the depth of 6 ml of the liquid.

Calculations

It has been shown by Kneen and Sandstedt (1941) and by the writers (unpublished) that the effects of alpha- and beta-amylase are additive in saccharification, even at relatively high ratios of beta- to alpha-amylase. This being the case, it is possible to determine accurately the alpha-amylase by suitable dextrinization measurements and apply a correction for its action on soluble starch in the malt saccharification (diastatic power) determination. That is, the dif-

[4] Presented in a paper given by Quick Landis at the 1943 Annual Meeting of the A.A.C.C.

ference between the diastatic power and the alpha-amylase saccharifying activity obtained by use of Table I is equal to the beta-amylase saccharifying activity.

Malt Saccharifying Activity (diastatic power): The malt saccharifying activity (diastatic power) is expressed in terms of maltose equivalent as follows:

$$\text{D.P. [maltose equivalent (dry basis)]} = \frac{(\text{blank-titration}) \times 144 \times 100}{(100 - \%\ \text{moisture})}$$

when 1 ml (0.05 g malt equiv.) extract is used in saccharification and 5 ml digest is used in the reducing power determination.

Alpha-amylase Saccharifying Activity: The saccharification due to alpha-amylase in the diastatic extract is given by

$$A = \frac{K \times 100}{(100 - \%\ \text{moisture}) \times \text{ml diastatic extract used in dextrinization}}$$

where

A = alpha-amylase saccharifying activity (maltose equivalent, dry basis)

and

K = the saccharification equivalent (maltose equivalent) of alpha-amylase dextrinization (reciprocal minutes). The values for "K" are obtained from Table I.

Beta-Amylase Activity: The difference between the malt-saccharifying activity, and the alpha-amylase saccharifying activity is equal to the beta-amylase activity.

$$i.e.,\quad B = \text{D.P.} - A$$

where

B = the beta-amylase activity [maltose equivalent (dry basis)].

A sample calculation using the above methods follows:

0.05*N* potassium ferricyanide reduced by 5 ml digest when 1 ml (0.05 g malt equivalent) diastatic extract is used in diastasis	=	5.00 ml
Moisture in malt sample	=	5.0%
Diastatic power $= \frac{5.00 \times 144 \times 100}{95.0}$	=	758 maltose equivalents
ml diastatic extract used in dextrinization	=	2 ml
Dextrinizing time (min to end point)	=	20 min
1/dextrinizing time = (1/20)	=	0.050

"K" value equivalent to 0.050 recip. min (Table I) = 75

Alpha-amylase saccharification $= \frac{75 \times 100}{95 \times 2}$ = 40 maltose equivalents

Beta-amylase activity = 758 − 40 = 718 maltose equivalents

This hypothetical malt would have an alpha-amylase activity of 40 maltose equivalents, beta-amylase activity of 718 maltose equivalents, and diastatic power of the sum of the two, or 758 maltose equivalents.

Summary

The original method of Sandstedt, Kneen, and Blish for alpha-amylase has been modified to make it more applicable to routine determinations in barley malt laboratories.

The dextrinization is measured at 20° instead of 30°C and the A.A.C.C. (Cereal Laboratory Methods) diastatic power procedure is substituted for the malt saccharifying activity determination of Kneen and Sandstedt.

The relation between alpha-amylase saccharifying and alpha-amylase dextrinizing activity has been established under these modified experimental conditions. By the use of the values so obtained, suitable corrections for alpha-amylase saccharifying activity can be made, and a valid measure of beta-amylase activity secured.

Two methods of preparation of enzymatically pure beta-amylase have been tested, and a simple method for ascertaining the presence of alpha-amylase described.

Acknowledgments

The encouragement and cooperation of Eric Kneen, members of the Malt Analysis Committee of the American Association of Cereal Chemists, and members of the Diastatic Power Committee of the American Society of Brewing Chemists, and others have aided greatly in the completion of the work reported here. Mrs. G. B. Alexander cooperated in the necessary laboratory work.

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A STUDY OF SOME OF THE VITAMIN B-COMPLEX FACTORS IN RICE AND ITS MILLED PRODUCTS, II

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The riboflavin and biotin content of different varieties and milled fractions of rice have been investigated and the findings reported herein are complementary to the study of thiamine, nicotinic acid, pantothenic acid, and pyridoxine presented in an earlier account of these experiments (Williams, Knox, and Fieger, 1943).

Although the riboflavin content of a similar group of rice samples was investigated by Kik (1943), few figures on the biotin content of rice products have appeared. Cheldelin and Williams (1942), in their report on the B vitamin content of various foods, supplied data for only one rice product, a commercially processed and packaged breakfast cereal. Lampen, Bahler, and Peterson (1942) reported the biotin content of one sample of rice polish and one sample of a rice bran concentrate. Cheldelin, Woods, and Williams (1943) include findings for one sample of polished rice in their investigation of B vitamin losses upon cooking various foods.

Materials and Methods

The samples used for these experiments were the same ones analyzed in the first part of the study, already mentioned. They were divided into two main groups: (1) samples of brown rice of six typical Louisiana varieties (Blue Rose, Fortuna, Early Prolific, Nira, Rexoro, and American Pearl) collected to study differences among varieties; and (2) samples of milled fractions of three typical varieties (Blue Rose, Early Prolific, and Fortuna) collected to investigate differences in vitamin content of the various milled fractions.

As pointed out in the earlier publication, these milled fractions consist of the products obtained in the different steps of the milling process. As more and more of the outer coats of the rice grain are removed and the rice progresses from the original rough rice to the finished product, seven fractions are obtained: (1) brown rice, (2) first-break rice, (3) second-break rice, (4) brushed rice, and (5) finished rice. The outer coats of the grain appear at the end of the process as (6) rice polish and (7) rice bran.

The samples were analyzed by the following procedures:

1. Riboflavin—the Conner and Straub (1941) fluorometric method.
2. Biotin—the Shull, Hutchings, and Peterson (1942) microbiological assay procedure for biotin, as modified by Shull and Peterson (1943). The samples were hydrolyzed in normal sulfuric acid at 15 lb of pressure for 30 min.

Discussion of Results

Riboflavin. Remarkably close agreement was found between the figures given in Table I and those reported by Kik (1943). In many

TABLE I

COMPARISON OF THE RIBOFLAVIN AND BIOTIN CONTENT OF THE MILLED FRACTIONS OF SEVERAL VARIETIES OF RICE

Variety	Number of samples brown rice	Number of samples each other fraction	Milled fractions [1]						
			Brown rice	1st break rice	2nd break rice	Brushed rice	Finished rice	Bran	Polish
			μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
RIBOFLAVIN									
Blue Rose	5	3	0.76	0.48	0.49	0.40	0.29	2.12	1.65
Fortuna	7	3	0.52	0.45	0.29	0.25	0.23	1.83	1.92
Early Prolific	5	3	0.56	0.34	0.20	0.18	0.20	2.48	1.77
Average			0.61	0.42	0.33	0.28	0.24	2.14	1.78
BIOTIN									
Blue Rose	5	3	0.114	0.061	0.059	0.053	0.050	0.541	0.543
Fortuna	7	3	0.123	0.056	0.050	0.051	0.034	0.485	0.833
Early Prolific	5	3	0.126	0.082	0.059	0.048	0.043	0.377	0.591
Average			0.121	0.066	0.056	0.051	0.042	0.468	0.656

[1] Measurements are for dry weight.

cases, values for the same milled fraction were within 5 to 10% of each other. In both cases, the fluorometric method of analysis was used.

The decrease in vitamin content with the increase in milling was illustrated by the riboflavin values, just as it has been by the data published in 1943. The sharpest drop in riboflavin content occurred between the brown rice and first break.

When compared with other foods, brown rice, polish, and bran do not rank very high in riboflavin content. White and yellow corn contain more riboflavin per unit weight than does brown rice, while wheat germ and wheat bran considerably exceed rice polish and rice bran in riboflavin content (Conner and Straub, 1941).

Biotin. Some difficulty was experienced with the biotin assay. The first hindrance was encountered in the preparation of the yeast filtrate, although the Shull and Peterson (1943) modified procedure was followed. It was found that warming the various solutions containing norit by means of a controlled bath alone did not produce a heat penetration sufficiently rapid for successful adsorption or elution, as the case might be. Heating to 55–60°C by means of a hot plate prior to placing the solution in a water bath, however, provided sufficient heat penetration and produced a highly satisfactory yeast filtrate.

A second difficulty was encountered as a result of anomalous values obtained in the assays of rice polish, rice bran, and brown rice. Similar drifts have been observed in the assays of cereal products as reported by Strong and Carpenter (1942) and Neal and Strong (1943). The effect produced most markedly by rice polish is probably one of stimulation as well as one of inhibition. The drift effect was obviated by reading samples at dilutions giving agreement within 10% for assays dosed at increasing levels of concentration. An assay of defatted rice polish indicated that the interfering substance was not in the fat fraction. Further work is being continued on this phase of the problem.

Although the biotin values reported in the present study were somewhat higher than any for rice samples published by Lampen, Bahler, and Peterson (1942), Cheldelin and Williams (1942), or Cheldelin, Woods, and Williams (1943), it should be pointed out that these workers reported values for only one sample in each case. Average values on the air-dry basis obtained in the present work, furthermore, agreed within 5% for brown rice and within 2% for rice polish with values obtained by workers at the Department of Biochemistry, University of Wisconsin, for brown rice and rice polish samples furnished by this laboratory. All samples here reported were checked against a control supplied by that laboratory.

TABLE II

COMPARISON OF THE RIBOFLAVIN AND BIOTIN CONTENT OF SEVERAL VARIETIES OF BROWN RICE

Variety	Number of samples	Vitamin content	
		Riboflavin	Biotin
		μg/g dry wt	*μg/g dry wt*
Blue Rose	5	0.76	0.114
Early Prolific	5	0.56	0.126
Nira	3	0.53	0.124
Rexoro	5	0.54	0.133
American Pearl	2	0.49	0.117
Fortuna	7	0.52	0.123
Composite average		0.57	0.121

The biotin values in Table I corroborated the trends exhibited by the other vitamin values, the vitamin content decreasing with increasing milling. Rice polish and bran were high in biotin content when compared with other foods (Lampen, Bahler, and Peterson, 1942).

The results reported in Table II do not show appreciable differences among the different varieties. The trend for the long-grained varieties, such as Fortuna and Nira, to lead in vitamin content was not demonstrated so clearly as in the earlier half of the present study.

Summary

Samples of different milled fractions of several varieties of rice were analyzed for riboflavin and biotin. Values obtained for riboflavin agreed closely with those of other workers; biotin values were somewhat higher than the few figures for isolated samples reported by other workers, but appeared to be in agreement with assays obtained at another laboratory.

All samples of milled fractions showed that the biotin and riboflavin content of the rice samples decreased with increased milling, as has been demonstrated for thiamine, nicotinic acid, pantothenic acid, and pyridoxine in the first part of the present study. In general, 66 to 75% of all six of the vitamins studied is removed in the milling process, and polished rice contains only about 12% of the vitamin content of rice polish.

No appreciable differences in riboflavin and biotin content were found among the several varieties.

Acknowledgment

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SIMPLIFICATION OF THE THIOCHROME METHOD FOR THIAMINE DETERMINATION

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With the widespread enrichment of flour there has come a need for a rapid yet reliable technique for the determination of thiamine in mill products and in bread. The thiochrome procedure given in Cereal Laboratory Methods (4th ed., 1941) appears to have been satisfactory in most respects but it is too time-consuming to be entirely useful as a routine control method. Several suggestions have previously been made as to means of simplifying the original thiochrome technique. Andrews and Nordgren (1941) found that the method could be much shortened by omitting the purification step of adsorption on zeolite. In their simplified method the sample is merely shaken with a 25% solution of potassium chloride, in dilute acetic acid, filtered, and an aliquot then oxidized in the usual manner. This technique, while apparently satisfactory for patent and first clear flours, has not been recommended for use with other products. Their studies would indicate, however, that various cereal products may be analyzed without the use of zeolite adsorption. Hoffer, Alcock, and Geddes (1943) studied a modification of the simplified procedure of Andrews and Nordgren and found that it was necessary to apply correction factors depending upon the product being analyzed.

The present paper describes a reasonably rapid method which can be applied to a wide variety of cereal products. As with other short methods, the zeolite adsorption has been eliminated in our modification. A single sample can be analyzed in 1 hr and one operator can analyze nine samples in 2½ hr with ease. The procedure is exactly the same for all mill products, and no correction factors need be used. In the case of bread analyses, the time required is somewhat longer; in our experi-

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ence a digestion period of 2 hr is required to complete the breakdown of cocarboxylase, which extends the time required for analyses by 90 min.

Proposed Simplified Thiochrome Method

Apparatus

(1) Fluorophotometer
(2) Centrifuge and 50-ml centrifuge tubes
(3) Erlenmeyer flasks, 250 ml
(4) Boiling water bath
(5) Cold-finger condensers
(6) Incubator, set at 50° C
(7) Test tubes, 50-ml
(8) Glass-stoppered shaking bottles, 25-ml

Reagents

(1) Acetic acid solution, 2%
(2) Sodium hydroxide, 1*N*
(3) Takadiastase solution, 6%, made up fresh daily
(4) Potassium ferricyanide solution, 1%. If kept in a dark bottle away from light this solution will last a week or more.
(5) Quinine sulfate solution. Dissolve sufficient quinine sulfate in 0.1*N* sulfuric acid to give a solution containing 1 μg quinine sulfate per ml
(6) Sodium hydroxide, 15%
(7) Isobutanol. This reagent should not give a blank reading above 4 units. Used isobutanol can be reclaimed by treatment with activated carbon (Pader, 1943)
(8) Standard thiamine solution. Make up in 2% acetic acid, a stock solution containing 10 μg thiamine per 25 ml. Store in a cold place away from light
(9) Potassium chloride, crystals, C.P.
(10) Sodium sulfate, anhydrous, technical grade

Procedure

Preparation of Extract. The weight of sample taken for extraction should be varied according to the expected thiamine content. The quantities shown below are recommended.

Expected thiamine content	Sample weight
mg/lb	*g*
0 –1.0	4.0
1.1–2.5	2.0
2.6–5.0	1.0
Over 5.0	0.5

Place the selected quantity of the material to be tested in a 250-ml Erlenmeyer flask, add 40 ml of 2% acetic acid, shake, insert cold-finger condenser, and place in boiling water bath for 15 min. Remove flask and place in cold water until the temperature of the solution drops to approximately 50°C. Add 5 ml of 1*N* NaOH, shaking solution during addition. Add 5 ml of 6% takadiastase solution, stopper flasks, and place them in an incubator at 50°C. After 30 min filter through Whatman No. 1 or equivalent paper. Pipette a 5-ml aliquot of the solution into a shaking bottle. The extract is now ready for oxidation.

Subject to the same treatment a control solution containing 1 μg of thiamine per 5 ml of solution. To 25 ml of the thiamine stock solution, add 15 ml of 2% acetic acid, 5 ml of NaOH, and then 5 ml of takadiastase solution. Place in incubator for 30 min, filter, and pipette a 5-ml aliquot into a shaking bottle.

Oxidation. It is usually convenient to oxidize two samples at a time. To each sample add approximately 0.5 g of KCl crystals and shake vigorously for a few seconds. Add 0.1 ml (2 drops) of 1% $K_3Fe(CN)_6$ solution and shake again. Add 3 ml of 15% NaOH solution and shake a third time. Add 15 ml of isobutanol, stopper, and shake vigorously for 1 min. Pour into a 50-ml centrifuge tube and centrifuge until any emulsion is broken. Separate the two layers and pour the alcohol phase into a test tube. Add enough anhydrous sodium sulfate to remove any remaining water and shake until a clear solution is obtained. Pour into a clean dry cuvette and measure fluorescence in the fluorophotometer which has previously been set with the quinine sulfate standard. The reading obtained is proportional to the amount of thiamine present in the sample plus any nonthiamine fluorescent impurities.

Blank Determination. Carry out a blank determination for each different type of product analyzed and for the control solution. To 5 ml of sample extract or of control solution add, in this order, KCl crystals, isobutanol, and NaOH. Stopper the bottle and shake for 1 min. Separate alcohol phase, dry, and measure the fluorescence as described above.

Calculation. Determine the amount of thiamine present from the following relation:

$$\text{Thiamine content, mg/lb} = \frac{R_x - R_{xB1}}{R_y - R_{yB1}} \times \frac{50}{5} \times \frac{1}{Z} \times 0.454, \text{ where}$$

R_x = Reading for unknown
R_y = Reading for Standard Thiamine Control Solution
R_{xB1} = Reading for blank carried out with unknown
R_{yB1} = Reading for blank carried out with thiamine control solution and
Z = grams sample taken

Accuracy of Simplified Method

In Tables I and II are given a summary of the results of the collaborative analysis of 127 samples of a variety of cereal products by five laboratories. The samples are grouped according to their approximate thiamine content because, as shown by Hildebrand and Geddes (1943), the experimental error of the thiochrome method increases with increasing thiamine level. Four of the five laboratories employed the

TABLE I

MEAN THIAMINE RESULTS OBTAINED BY REGULAR AND MODIFIED THIOCHROME PROCEDURES

Sample		Thiamine range	Regular method					Simplified method
Type	Number analyzed		A	B	C	D	Avg.	E
		mg/lb	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*
Short patent	20	0.15– 0.35	0.28	0.28	0.28	0.27	0.28	0.28
Long pat.; st. grade	21	0.50– 0.80	0.57	0.57	0.58	0.58	0.58	0.60
Durum; white rye	6	0.75– 1.50	1.06	1.03	1.09	1.01	1.05	1.04
First clear	17	1.30– 1.60	1.45	1.43	1.48	1.50	1.46	1.49
Enriched pat.	20	1.45– 2.25	1.78	1.72	1.75	1.82	1.77	1.84
Whole wheat	23	1.85– 2.35	2.15	2.15	2.13	2.06	2.12	2.15
Noodles; dk. rye; oat flr.	10	2.50– 5.50	3.39	3.41	3.40	3.38	3.40	3.37
Second cl.; red dog; germ	10	7.00–10.50	8.64	8.47	8.14	8.24	8.37	8.37
All samples	127	0.15–10.50	2.05	1.97	1.96	1.96	1.98	1.99

TABLE II

ERRORS OF REGULAR AND MODIFIED THIOCHROME PROCEDURES[1]

Sample		Regular method					Simplified method
Type	Number analyzed	A	B	C	D	Avg.	E
		mg/lb	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*
Short patent	20	0.042	0.043	0.031	0.020	0.034	0.021
Long pat.; st. grade	21	0.069	0.067	0.033	0.022	0.048	0.036
Durum; white rye	6	0.041	0.049	0.072	0.044	0.052	0.040
First clear	17	0.294	0.236	0.295	0.157	0.246	0.180
Enriched pat.	20	0.173	0.357	0.257	0.213	0.250	0.269
Whole wheat	23	0.376	0.354	0.308	0.315	0.338	0.274
Noodles; dk. rye; oat flour	10	0.984	0.486	0.964	0.737	0.793	0.856
Second cl.; red dog; germ	10	0.417	0.496	0.502	0.405	0.455	0.459
All groups	127	0.265	0.249	0.265	0.210	0.248	0.231

[1] Errors were calculated from the relation

$$E = \sqrt{\frac{(x_1 - m_1)^2 + (x_2 - m_2)^2 + \cdots}{N - 1}}$$

where

x_1, x_2, etc. = individual laboratory values for samples 1, 2, etc.
m_1, m_2, etc. = mean values, all laboratories, for samples 1, 2, etc.

and

N = number of samples.

thiochrome method essentially as described in Cereal Laboratory Methods (4th ed., 1941), while the values listed under "Simplified Method" are characteristic of the procedure described in this paper. It is evident that the accuracy of the modified method is entirely satisfactory.

Summary

A simplification of the thiochrome method for thiamine analysis is described. The short method effects a significant saving in time and effort, can be applied to all types of flour mill products and to many

other types of cereal products, and requires no correction factors. No changes need be made in the method except for the analysis of baked goods.

The accuracy of the simplified method, as shown by the collaborative analysis of 127 samples of various cereal products, compares favorably with that of the regular thiochrome method.

The simplified method is admirably suited to routine control determinations.

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THE COMPOSITION OF THE "AMYLODEXTRIN" FRACTION OF WHEAT FLOUR

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The so-called "amylodextrin" or "small-granule" fraction of wheat flour is of importance both in enhancing the baking properties of flour and in the commercial production of wheat starch. Sandstedt, Jolitz, and Blish (1939) have reported that this fraction contributes to the handling characteristics of dough and is essential for the production of bread with a tender crumb. In the production of wheat starch, the "amylodextrin" or "tailings" fraction has significance because it is an almost profitless by-product. In commercial operations, after removal of the starchy material from the gluten mass by washing with water, the starch is separated by tabling. The flocculent material of low density, that is "amylodextrin" fraction or tailings starch, which flows over the end of the table is recovered and converted into a low-grade and low-priced adhesive. Since this fraction may represent as much as 20% of the starch, information concerning its composition would be of use in the development of a more profitable outlet.

[1] This is one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Considerable confusion has arisen in the literature owing to the conflicting statements concerning the actual composition of the "amylodextrin" fraction of wheat flour. Sandstedt, Jolitz, and Blish (1939) considered it to be composed of dextrins produced by amylase activity. The presence of large numbers of small starch granules was reported by Goldbeck (1916) and by others. Pentosans represent 14% by weight of the small-granule fraction according to Baker, Parker, and Mize (1943), who regarded them as being adsorbed upon the relatively large surface area of the small granules. It was believed by these authors that the coating of pentosan on the starch granules might account for many of the properties of the fraction.

In the present investigation, microscopic and analytical methods have been used to determine the composition of the "amylodextrin" fraction.

Experimental

Preparation of Fractions. Two 1941 crop year wheats were used for this study (which was carried out chiefly in 1942): (1) Turkey hard red winter, grown at Manhattan, Kansas, graded No. 1 dark hard, and (2) Trumbull soft red winter, grown at Wooster, Ohio, graded No. 1 red winter. Each contained only a trace of dockage which was removed before the wheat was weighed for use.

Starch and "amylodextrin" fractions were separated from three samples of each wheat: (1) whole wheat kernels, steeped 24 hr in distilled water at approximately 5°C, and subsequently ground twice through a Russwin No. 2 handmill, using the nut-butter cutter, (2) straight flour obtained by milling the wheat on a laboratory-model Buhler mill, and (3) flour obtained by remilling the original flour twice through the Buhler mill.

Each sample was extracted as follows: 400 g of ground sample (original air-dried weight) was worked into a stiff dough with distilled water and allowed to stand for 1 hr at room temperature to facilitate formation of a "gluten ball." Starch was extracted from the hydrated mass by working it in successive portions of distilled water. Fine fiber was removed by passing the combined extraction liquors, totaling 6 to 8 l in volume, through No. 13 standard bolting silk. The starch and "amylodextrin" fractions were centrifuged from the liquid and washed once with distilled water after which the upper, "amylodextrin" layer was carefully removed from the starch with a spatula. The starch was resuspended in distilled water and recentrifuged, the residual traces of the "amylodextrin" fraction being removed as before. The remaining starch was washed twice with distilled water and air-dried at room temperature. Since the "amylodextrin" layer became horny when dried from water, it was, except during drying studies and for analysis,

air-dried at room temperature after removal of water by three or more acetone washes.

Analytical Methods. The composition of the starch and "amylodextrin" fractions was determined by standard analytical procedures. The moisture in the "amylodextrin" and starch fractions was determined by drying to constant weight at 100°C over P_2O_5 in an Abderhalden drier; in wheat, by drying the ground grain for 1½ hr in a Brabender Moisture Tester at 130°C. Determinations were made for nitrogen by the Kjeldahl-Gunning-Arnold method (A.O.A.C., 1940, p. 26), starch by a diastase method (A.O.A.C., 1940, p. 359), and fatty acids or fats by acid hydrolysis and extraction from the hydrolysate. Pentosans were determined as furfural by a modified method developed by the Analytical and Physical Chemical Division of the Northern Regional Research Laboratory. The distillate obtained by the official A.O.A.C. (1940) procedure was redistilled and the furfural was precipitated by thiobarbituric acid. In this way erroneously high results due to measurement of the hydroxymethylfurfural formed from hexose material were avoided.

Examination of Starch and "Amylodextrin" Fractions. Starch and "amylodextrin" fractions prepared from whole wheat by wet milling, and from once- and thrice-milled flour were examined microscopically, analytically, and from the standpoint of yields.

More starch was recovered from wheat flour than from whole wheat because of the larger percentage of starch present in the former. The yield of starch fraction decreased upon remilling the flour from soft wheat, but was not significantly affected by the remilling of hard wheat flour (Table I). Photomicrographs of starch fractions from Turkey wheat are shown in Figure 1; the fractions from Trumbull wheat had a similar microscopic appearance. Starch fractions obtained from the various raw materials showed no unusual characteristics or differences. In each case, the nitrogen content of the starch was normal, and pentosans were absent. More small granules were freed by milling through the Buhler mill than by use of the handmill (Figs. 1A and 1B). No appreciable granule damage was observed (Figs. 1A, 1B, and 1C); birefringence was clear (Fig. 1D).

"Amylodextrin" fractions, on the other hand, varied considerably with the milling method, the yield increasing significantly with increasing severity of grinding. (See Table I.) Photomicrographs of these fractions are shown in Figure 2. These fractions were found to be composed primarily of very small starch granules, their average size being comparable to that of the smallest granules in the starch fraction (Figs. 2A, 2B, and 2C). In addition, large granules were present in varying amounts. From their partial or complete loss of birefringence

(Fig. 2D) and their ready absorption of stains, such as benzopurpurin or congocorinth, it was apparent that practically all the larger granules had suffered damage and, in consequence, were partly, often completely, gelatinized in the liquid in which they were mounted for observation. The number of damaged granules increased with severity

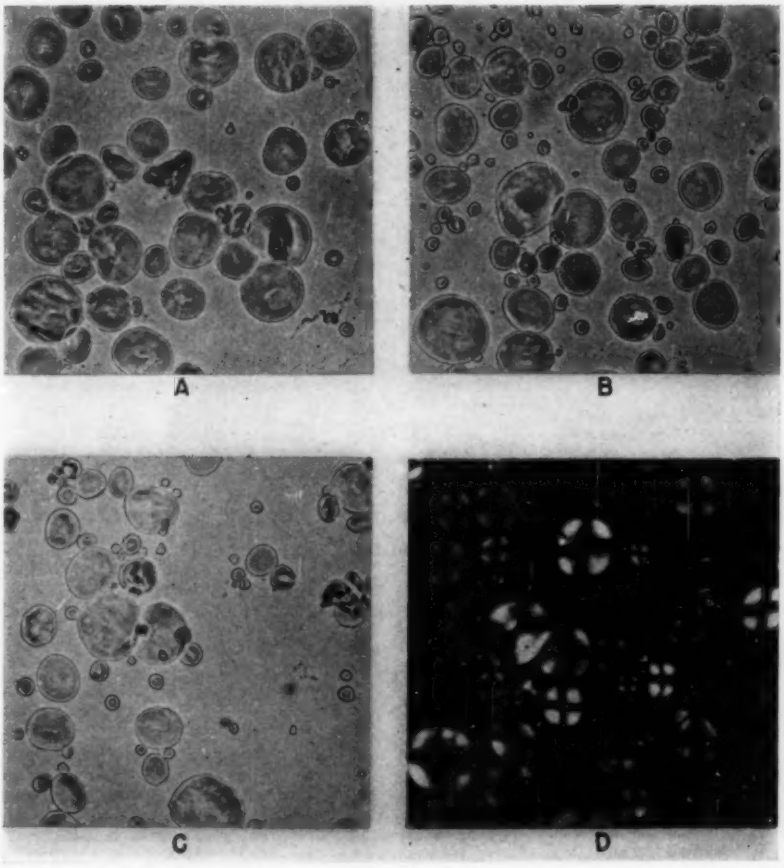

Fig. 1. Starch from Turkey wheat. (Magnification 285 ×)
A. Extracted after wet milling.
B. Extracted from flour.
C. Extracted from twice remilled flour.
D. Same field as B, between crossed nicols.

of grinding, being least when the handmill was used and most in the twice-remilled material (Figs. 2A and 2C). All the "amylodextrin" fractions included small gluten masses and relatively large fragments, from the microscopic standpoint, of cell debris.

The analytical data confirmed the microscopic evidence that the

"amylodextrin" fraction was composed largely of starch, which, in all cases, comprised 87 to 94% of the fraction. Nitrogen analyses showed the presence of from 0.9 to 1.8% protein ($N \times 5.7$). Protein increased with the extent of milling in the case of hard wheat, but fluctuated less in the case of soft wheat.

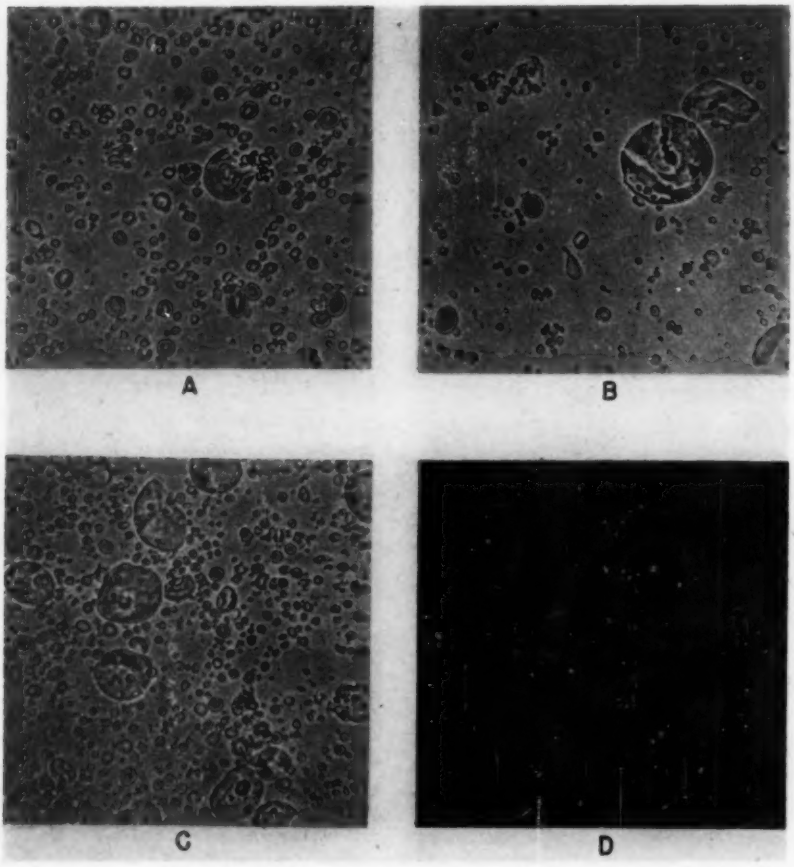

Fig. 2. "Amylodextrin" fraction from Turkey wheat. (Magnification 285 ×)
A. Extracted after wet milling.
B. Extracted from flour.
C. Extracted from twice remilled flour.
D. Same field as C, between crossed nicols.

A more complete analysis of a typical "amylodextrin" fraction is shown in Table II. The major nonstarch materials were pentosans, protein, fatty acid, and ash. Less than 3.5% of the fraction was unidentified and this is believed to be essentially cellulosic material in the cell wall fragments. A cellulose determination of so small an

TABLE I

YIELD OF AND ANALYTICAL DATA ON STARCH AND "AMYLODEXTRIN" FRACTIONS

All data on dry basis

Sample	"Amylodextrin" fraction			Starch fraction	
	Yield	Nitrogen	Starch	Yield	Nitrogen
	%	%	%	%	%
Turkey, whole wheat	9	0.16	94.6	50	0.04
Turkey, flour	10	0.25	91.1	60	0.05
Turkey, twice remilled flour	15	0.32	89.6	59	0.05
Trumbull, whole wheat	8	0.32	87.5	49	0.04
Trumbull, flour	12	0.24	90.1	67	0.05
Trumbull, twice remilled flour	15	0.29	89.0	60	0.05

TABLE II

COMPOSITION OF "AMYLODEXTRIN" FRACTION FROM TURKEY WHEAT FLOUR (ON DRY BASIS)

Starch	Pentosans	Protein ($N \times 5.7$)	Fatty acids	Ash
%	%	%	%	%
90.1	4.0	1.5	0.7	0.27

amount would not have been practical, however, and was not attempted. Rough microscopic estimation suggested that the amount of cell debris present was approximately of the same order as the pentosans found by analysis. That the pentosans were largely in the cell debris was further supported by the fact that the starch fraction, which contained no cell wall material, also contained no pentosans. The fatty acid content of the fraction was about the same as that of prime-quality starch (0.7%), and, presumably, the fat was present mainly as a component of the starch granules. The ash, however, was twice as great as that of ordinary wheat starch.

The most noticeable characteristic of the small-granule fraction is its semifluid nature. Density determinations made by pycnometer measurements gave:

"Amylodextrin" fraction	1.453
Methanol-extracted "amylodextrin" fraction	1.463
Starch fraction	1.485

The effect of treating the small-granule fraction with amylase was investigated in order to determine if this treatment facilitated the preparation of the small-granule fraction in a pulverulent form, the development of such a procedure being of value in the commercial processing of flour for starch. Removal of the gelatinized damaged granules by enzyme treatment only slightly lessened the semiliquid

nature of the fraction. On drying the treated fraction in air, a horny intractable product was obtained. Apparently the pentosan and protein components, rather than gelatinized starch granules of the fraction, are mainly responsible for the great difficulty in obtaining the fraction as a dry, friable powder by ordinary dehydration.

Discussion

In this work it has been shown that the small-granule or "amylodextrin" fraction comprises starch (87–94%), protein (1–2%), pentosans (4%), fatty material (about 0.7%), and ash (about 0.3%). In addition there is a small amount of cellulosic material, probably about 3%. The starchy material is composed of ungelatinized small granules and damaged, large granules which gelatinize partially or wholly in cold water, the former predominating. The observations on the presence of small granules confirm the work of Goldbeck (1916) and others. The amounts of protein and fatty material found in the small-granule fraction are approximately in agreement with values previously reported by Sandstedt, Jolitz, and Blish (1939). Data on pentosan content, however, are of a different order than those given by Baker *et al* (1943), who reported 14% as compared to about 4% found in this work. It is difficult to explain this discrepancy. Baker *et al* do not give the starch content of their "amylodextrin" fraction which was extracted with a salt solution. Since the complete analysis is not available, it is impossible to judge whether their "amylodextrin" fraction was dissimilar to those studied by the present writers.

An outstanding characteristic of the small-granule fraction is its semifluid nature, which cannot be attributed to density alone. The large difference in volumes (per unit weight of dry substance) of the starch and "amylodextrin" layers after centrifuging indicates a difference in the packing of the particles. After centrifuging, a layer of ordinary starch is somewhat plastic (essentially solid) indicating close packing of the granules, whereas the layer of small-granule starch is viscous (essentially fluid) indicating extremely loose packing. Other starches consisting of small granules tend to have similar characteristics. This may be due to the much larger ionic charge per unit volume that small granules possess, the effect of a charge on a particle becoming more pronounced as the size of the particle decreases and its surface area per unit volume increases. The high ash content of the small-granule fraction suggests that a considerable part of the inorganic matter may be adsorbed by the particles, thus increasing their polarity. Particle size is not, however, the sole factor influencing consistency. Rice starch, with granules approximately equal in size to the small granules of the "amylodextrin" fraction, is less fluid than the latter,

but more fluid than prime-quality corn or wheat starch. Further work would be necessary to determine whether small starch granules of different species exhibit variations in packing owing to differences in shape, etc. The low density and large area of the pieces of cell debris probably also contribute materially to the physical nature of the fraction as a whole.

It would appear desirable to avoid designation of the small-granule fraction by the name "amylodextrin." Amylodextrin is a recognized enzymic degradation product of starch. Unlike the small-granule fraction, it is water soluble. The name "small-granule fraction" has a disadvantage in that important properties of the material appear to be due to components other than the small granules. "Tailings fraction" might be a more acceptable term, since the fraction separated by centrifuging is essentially the same as that flowing over the tail when crude starch is tabled, and since the name has no implications as to chemical composition.

Summary

The so-called "amylodextrin," small-granule or tailings fraction separated by centrifuging crude wheat starch after the removal of the bran and gluten, is composed of very small starch granules, varying amounts of damaged large granules (depending upon severity of milling), and smaller quantities of protein, fatty acids, ash, and pentosans, and cellulose in the cell wall debris. The damaged granules gelatinize in cold water. There is evidence of relatively little degradation of the starch.

Large sorptive surfaces of the small granules, together with high ash content, suggest that the semifluid consistency of the fraction is due at least in part to an electroviscous effect.

Acknowledgments

Wheat samples were collected and dry milled by the Commodity Development Division, and starch, nitrogen, ash, pentosan, and fatty acid determinations were made by the Analytical and Physical Chemical Division, of this Laboratory. The assistance of C. W. Bice, J. W. Eck, and H. H. Rhodes in the laboratory experiments is gratefully acknowledged.

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REPORT OF 1943–44 COMMITTEE ON RELATION OF LABORATORY BAKING TO SHOP PRACTICE

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(Read at the Annual Meeting, May 1944)

Six samples of commercially acceptable bread flour, as listed in Table I, were submitted to seven collaborators with a request to bake and evaluate them in accordance with their existing methods and report the following information for each sample: (1) mixing time and type of mixer, (2) fermentation time and yeast percentage, (3) fermentation temperature, (4) absorption (13.5% moisture basis), (5) proof time and temperature, (6) baking time and temperature, (7) loaf volume, (8) scaling weight of loaf, (9) weight of loaf from oven. Identical data on the same samples were obtained from commercial shops.

TABLE I

FLOURS EMPLOYED IN COLLABORATIVE STUDY

Sample No.	Flour type	Protein[1]	Ash[1]
		%	%
1	Spring	12.2	0.40
2	Spring	12.3	0.42
3	Winter	11.9	0.39
4	Winter	12.4	0.44
5	Spring	12.5	0.46
6	Winter	12.4	0.47

[1] 13.5% moisture basis.

The baking methods used by the various collaborators covered a rather wide range of testing procedures. Two laboratories used both sponge and straight dough methods of baking, one used the sponge procedure exclusively, while the remainder used straight dough methods exclusively. Three operators used the "one hundred-gram" loaf test, while the remainder baked one-pound loaves. Six collaborators used variable absorption with one employing a fixed absorption; four employed varying mixing times with three using a constant mixing time for all flours. Six of the laboratories were equipped with Hobart-Swanson type mixers or the McDuffy bowl in connection with a Hobart mixer; the remaining collaborator employed a Washburn Crosby type mixer. Four varied fermentation and proofing times, while three held these constant. All collaborators used the same fermentation and proofing temperatures.

The mixing times are recorded in Table II. Collaborators 4, 5, 6, and 7 all employed a mechanical means of testing the flours for

TABLE II
MIXING TIMES

	Sample No.					
	1	2	3	4	5	6
Col. 1	8	8	8	8	8	8
Col. 2	3	3	3	3	3	3
Col. 3	3	3	3	3	3	3
Col. 4	4	3½	2½	2½	4	3
Col. 6	3	3	2	2¼	3¼	2½
Col. 7	3½	3⅝	2¼	2½	4	2⅞
4 Sponge	3½	3	2	2	3½	2½
5 Sponge	6	6	4	4	6	6
6 Sponge	3½	3	2¼	2¼	3½	3
Commercial	8½	8½	9	9	8½	9

mixing time. Collaborator 5 used a fixed time only in the dough stage of his sponge and dough procedure, and the time listed for this operator is that at which his best results were obtained. Where mixing time was varied, there was rather close agreement between operators. Increased mixing time for the commercial shop on samples 3 and 4 is due to the softer dough as noted in Table III. A definite tendency toward longer mixing time for the spring wheats is also evident.

TABLE III
ABSORPTIONS (13.5% moisture basis)

	Sample No.					
	1	2	3	4	5	6
Col. 1	64	64	64.5	64.5	64.5	
Col. 2	59	59	59	59	59	59
Col. 3	62.3	62.5	64.9	65.2	61.8	64
Col. 4	63	62.5	65	66	62.5	63
Col. 6	64.2	64.8	64.8	64.6	64.8	64.8
Col. 7	63.5	61	61.5	62	64	63
4 Sponge	64.5	64.0	66.5	67.5	64	64.5
5 Sponge	62	62	64	63	63	63
6 Sponge	64.5	65	65	64.8	65	65
Commercial	63.5	63.2	62	65	63.5	63.2
Average	63.0	62.7	63.9	64.0	63.1	63.2

The absorption data are given in Table III. Collaborator 3 employed a farinograph to determine absorption, while the others relied on feel and experience, and it is quite evident that the personal equation has markedly influenced the results. The average absorption values of all collaborators follow the same trend as the commercial figures. The winter wheats were given slightly higher absorption than spring wheats.

TABLE IV

FERMENTATION TIME IN MINUTES

	Sample No.					
	1	2	3	4	5	6
Col. 1	240	240	270	240	240	
Col. 2	180	180	180	180	180	180
Col. 3	180	180	180	180	180	180
Col. 4	113	111	106	108	111	110
Col. 6	180	172	142	142	180	146
Col. 7	180	180	180	180	180	180
4 Sponge	260	260	260	260	260	260
5 Sponge	340	340	340	370	370	340
6 Sponge	270	270	250	250	270	250
Commercial	270	260	225	215	240	360

The various fermentation times employed are recorded in Table IV. Three collaborators did not vary their fermentation, while the remainder showed some range of fermentation. Because of the wide range in the quantity of yeast employed, a second table has been prepared showing the fermentation times in minutes per percent of yeast (Table V).

TABLE V

FERMENTATION TIME IN MINUTES PER PERCENT YEAST

	Sample No.					
	1	2	3	4	5	6
Col. 1	101	101	114	101	101	
Col. 2	72	72	72	72	72	72
Col. 3	60	60	60	60	60	60
Col. 4	56.5	55.5	53	54	55.5	55
Col. 6	75	71	59	59	75	60
Col. 7	90	90	90	90	90	90
4 Sponge	87	87	87	87	87	87
5 Sponge	122	122	122	128	128	122
6 Sponge	78	78	69	69	78	69
Commercial	78	75	73	66	78	X

Employing the sponge method, collaborator 6 adopted a fermentation time which agrees rather closely with that used by the commercial shop. The others who used variable fermentation times all gave the spring wheat flours more fermentation than the winter wheat flours. This is true also in the commercial tests. These results indicate that the fermentation times employed by an individual laboratory might correlate satisfactorily with those of a given commercial bakery.

TABLE VI

PROOF TIME

	Sample No.					
	1	2	3	4	5	6
Col. 1	58	58	57	58	58	
Col. 2	40	40	40	40	40	40
Col. 3	55	55	55	55	55	55
Col. 4	54	54	55	53	52	48
Col. 6	65	65	64	63	62	60
Col. 7	55	55	55	55	55	55
4 Sponge	49	50	47	48	45	42
5 Sponge	70	72	70	69	65	58
6 Sponge	67	68	70	68	65	58
Commercial	60	59	55	58	60	

The proof times recorded in Table VI show less variation between operators than the data for mixing and fermentation times. Where proof time was varied there was a trend toward shorter proofing time with the samples having the highest protein.

TABLE VII

LOAF VOLUME IN CC/G OF DOUGH

	Sample No.					
	1	2	3	4	5	6
Col. 1	5.16	5.13	5.12	4.97	5.02	
Col. 2	4.15	4.05	3.94	4.19	4.45	4.28
Col. 4	4.25	4.27	4.20	4.25	4.25	4.22
Col. 6	5.80	5.90	5.6	5.50	5.78	5.65
Col. 7	4.94	4.91	4.79	4.89	5.31	4.86
4 Sponge	4.39	4.40	4.39	4.52	4.52	4.35
5 Sponge	4.89	4.92	4.72	4.79	4.75	4.75
6 Sponge	5.90	6.0	5.7	5.62	5.85	5.72
Avg.[1]	4.86	4.85	4.73	4.76	4.96	4.75
Avg.[2]	5.06	5.10	4.89	4.97	5.04	4.94
Commercial	4.59	4.55	4.48	4.60	4.68	4.53

[1] Average straight dough.
[2] Average sponge dough.

The loaf volumes expressed on a unit weight of dough are presented in Table VII. The average loaf volumes for all collaborators rank the flours in the same order as their protein content.

The results of this study serve to emphasize some of the difficulties involved in attempting to correlate laboratory baking results with shop practice. The problem of correlating the absorption and mixing time used in the laboratory with shop practice is relatively simple, since instruments such as the micromixer and farinograph are available

for these purposes. It would seem necessary to employ a fixed formula, preferably that of the baker, and vary the fermentation to obtain either an optimum loaf or one that corresponds to that produced under shop conditions. Proof time could either be held constant or varied to give a loaf having a similar specific volume to that produced commercially. Studies of the relation between experimental and commercial baking results should be continued. The ultimate goal can best be achieved by complete cooperation between the cereal chemist and the practical baker. It is therefore suggested that consideration be given to the possibility of further studies being undertaken as a cooperative effort of the cereal chemists and the bakery engineers.

Acknowledgments

The members who collaborated in this work were: Ray Bohn, Karl Finney, E. N. Frank, J. Freilich, J. H. Lanning, R. W. Mitchell, Ray Powers, and D. B. Pratt.

THE RELATIONS BETWEEN KJELDAHL PROTEIN, ZELENY PROTEIN, AND LOAF VOLUME IN WESTERN CANADIAN WHEATS AND FLOURS[1]

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(Presented at the Annual Meeting, May 1944; received for publication May 26, 1944)

About four years ago, Zeleny (1941) introduced a new simple procedure for determining protein content in wheat and flour. The proteins are peptized in a dilute alkaline solution, a stable colloidal suspension of the proteins is produced by a controlled change of the hydrogen-ion concentration, and the resulting turbidity is measured in a photometer by transmitted light. Zeleny found a close association for flours between light transmission and protein not peptized by 5% potassium sulfate solution, and Zeleny, Neustadt, and Dixon (1942) also found a close association for wheats between light transmission and a calculated value for endosperm protein. For these reasons they stated that ". . . the results obtained are probably a somewhat better index of baking quality than are the values obtained by the conventional Kjeldahl procedure."

This hypothesis and the simplicity of the method suggested that further investigation might prove profitable. Accordingly, a study was made of the relations between loaf volume, Zeleny protein, and

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Kjeldahl protein. Baking strength, as measured by loaf volume, is only one of the components of bread-making quality in flour; but it is an important one, which also lends itself to quantitative study. Although no closer relation was found between Zeleny protein and loaf volume than between Kjeldahl protein and loaf volume, the results of the study are of sufficient interest to warrant publication.

Materials and Methods

When assessing the ability of any method for estimating loaf volume, it is advisable to investigate both inter- and intravarietal relations. For this purpose, the ideal series of samples comprises a number of different varieties, each grown at a number of different stations. Unfortunately it was not possible to obtain such a series, and a compromise had to be made by using one series of samples to study intervarietal relations and another to study intravarietal relations.

The first series consisted of composites of 25 varieties that had been grown in the "Uniform Variety Tests" by the Dominion Department of Agriculture at nine different stations in Western Canada during 1943. The second series consisted of 50 samples of Thatcher wheat grown by registered seed-growers at 50 different points throughout Western Canada in the same year. All samples graded 3 Northern or higher.

The wheats, which were cleaned free from dockage, were milled in an Allis-Chalmers experimental mill, and a standard feed flour was used to control the yields. Flours of approximately 71% extraction were obtained and these were baked in triplicate by the standard malt-phosphate-bromate formula.

Protein content was determined in duplicate on duplicate subsamples of the wheats, and in quadruplicate on the flours, by the Kjeldahl-Gunning-Arnold method. Corresponding replicate determinations of light transmissions were made by the procedure outlined by Zeleny *et al* (1942), with the use of an Evelyn colorimeter and a light filter of 540 mμ. However, instead of shaking the samples and the peptizing agent intermittently for 3 min, they were rotated 50 to 60 times per min for 5 min.[2] The wheats for the Kjeldahl method were ground through a burr mill with a close setting, and for the Zeleny method they were ground through a Wiley mill with a 1-mm sieve. The Kjeldahl proteins were corrected to a 13.5% moisture basis, and Zeleny transmission values were obtained on the same basis by appropriate adjustment of the amount of sample weighed out.

[2] This change was suggested in a private communication from Dr. L. Zeleny who pointed out that vigorous shaking appears to have some undesirable effect on the physical properties of the gluten and tends to cause somewhat erratic results.

Results and Discussion

Relations with Loaf Volume. The primary object of the investigation was to determine whether or not the Zeleny method gives results that are more closely related to loaf volume than those given by the Kjeldahl method. This matter can be examined with the aid of the scatter diagrams in Figures 1 and 2. The former shows that the range

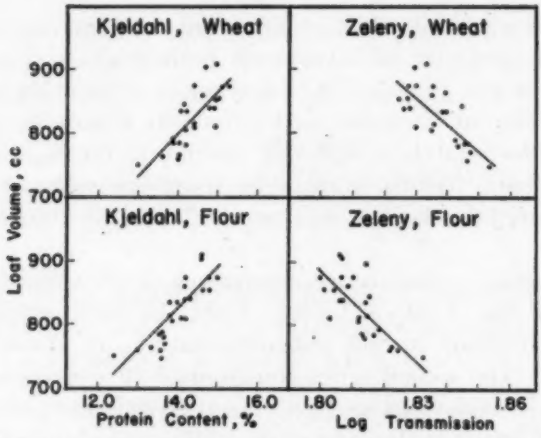

Fig. 1. Scatter diagram for the 25 varieties showing relations between Kjeldahl protein and loaf volume and Zeleny values and loaf volume.

of protein contents for the intervarietal series is quite narrow; but this is to be expected because the samples represent composites for varieties that are normal in protein content and that have been grown under the same environmental conditions. In the Thatcher series (Fig. 2) a comparatively wide range of protein contents is represented. It is hardly possible to tell by inspection of these figures whether the relations for Zeleny protein are closer than those for Kjeldahl protein, and the correlation coefficients must therefore be examined.

The simple correlation coefficients are shown in Table I. All are highly significant, but those for Zeleny protein and loaf volume are lower than those for Kjeldahl protein and loaf volume except in one instance in which they are equal. These differences, however, are not great enough to be statistically significant. Therefore, all that can be said is that the Zeleny method measures some fraction of the total protein that is no more closely related to loaf volume than is total protein determined by the Kjeldahl method.

As the Zeleny method measures some fraction of the total protein, and as this fraction is closely correlated with loaf volume, it is important to determine whether this relation is independent of bilateral

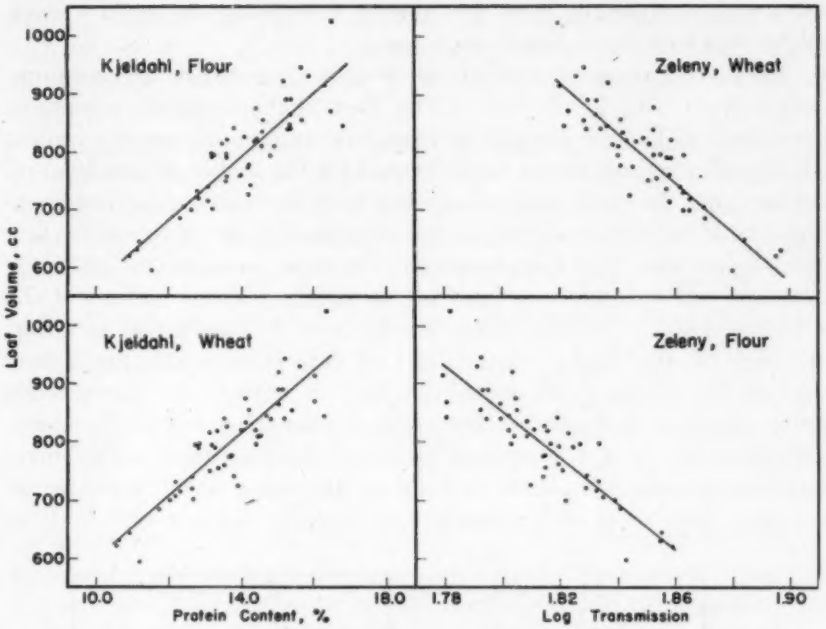

Fig. 2. Scatter diagram for Thatcher series showing relations between Kjeldahl protein and loaf volume and Zeleny values and loaf volume.

relations with total protein. Accordingly, the partial correlations of loaf volume with Zeleny protein independent of Kjeldahl protein were calculated. These partial correlations (Table I) are extremely small,

TABLE I

SIMPLE AND PARTIAL CORRELATION COEFFICIENTS FOR LOAF VOLUME WITH KJELDAHL PROTEIN AND WITH ZELENY PROTEIN

Series	Simple correlations: Loaf volume × Kjeldahl protein	Simple correlations: Loaf volume × Zeleny protein	Partial correlations: Loaf volume × Zeleny independent of Kjeldahl
25 Varieties			
Wheat	0.79 **	−0.75 **	−0.10
Flour	0.84 **	−0.78 **	−0.03
Thatcher			
Wheat	0.88 **	−0.88 **	−0.20
Flour	0.90 **	−0.88 **	0.24

Note: In this and subsequent tables, ** denotes 1% level of significance attained.

and thus tend to prove that the Zeleny method is measuring some moderately constant proportion of the total protein. Perhaps it should be emphasized here that these conclusions are based upon studies with wheats that contain few if any damaged kernels. Similar

tests with samples of light test weight containing damaged kernels might well lead to modified conclusions.

In this connection it is of interest to quote from a private communication from Dr. L. Zeleny: "The fact that globulins, albumins, proteoses, and other simpler nitrogenous compounds remain almost completely peptized in the medium used for the turbid suspension and the fact that the proteins of wheat bran have been shown to contribute but very little to the turbidity of the suspension make it appear reasonable to postulate that the photometric method is essentially, although probably not strictly, a measure of the gluten protein content of the wheat or flour. This hypothesis is supported by the further fact that although the Kjeldahl protein content of flour is invariably lower than that of the wheat from which the flour is milled, the photometric protein content of the flour is invariably higher than that of the wheat. Total protein (N × 5.7) is always lower in the flour than in the corresponding wheat, but gluten protein, on the other hand, is higher in the flour since most of the non-gluten proteins and but very little of

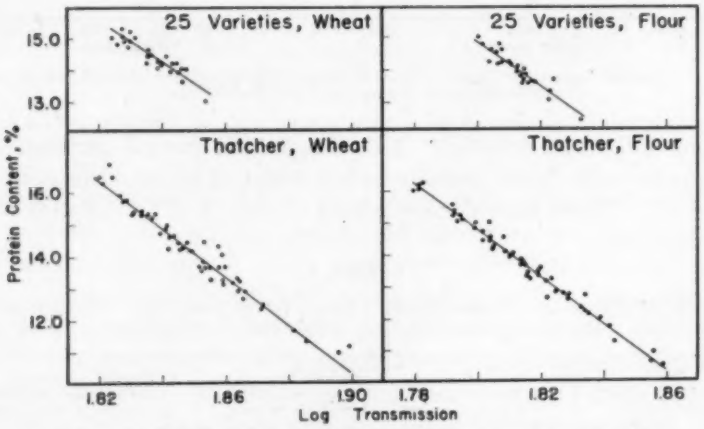

Fig. 3. Scatter diagram for the 25 variety and Thatcher series showing relation between Kjeldahl and Zeleny values.

the gluten protein are removed with the bran and shorts in the milling process." Figures 1 and 2 illustrate this last statement; for the Zeleny method flour proteins are much higher than those of the wheats from which they were milled (*i.e.* transmission values are lower for flours), whereas a slight difference, but in the opposite direction, is shown by the Kjeldahl data.

There are other comparisons of correlation coefficients that can be made from the data in Table I, but these are of secondary importance. The correlations for loaf volume and flour protein tend to be higher

than those for loaf volume and wheat protein, and the Thatcher correlations tend to be higher than the intervarietal correlations. These differences are in the expected directions, though they are not statistically significant.

Relation between Kjeldahl and Zeleny Protein. The data were also examined to determine whether the Zeleny method could be used to predict Kjeldahl protein. The scatter diagrams for the Zeleny-Kjeldahl relations are shown in Figure 3, and the corresponding correlation and regression coefficients are given below:

	Correlation coefficients		Regression coefficients	
	25 Varieties	Thatcher	25 Varieties	Thatcher
Wheat	−0.92 **	−0.98 **	−63	−76
Flour	−0.93 **	−0.99 **	−71	−74

All the correlation coefficients exceed the values required for the 1% level of significance, and indicate that Kjeldahl protein can be predicted from Zeleny protein with considerable precision. The standard errors of estimate from means of quadruplicate determinations are as follows: ± 0.2% protein for wheat and flour in the intervarietal series and for flour in the Thatcher series, and ± 0.3% protein for wheat in the Thatcher series. However, the same prediction equations cannot be used both within and between varieties. This follows from the fact that there are significant differences between the regression coefficients for the Thatcher and the intervarietal series. The statistics thus suggest that an accurate general equation for predicting Kjeldahl protein from Zeleny protein cannot be developed for a miscellaneous group of samples representing different varieties grown under various environmental conditions. Satisfactory prediction will probably be limited either to samples of one variety grown in different places, or to samples of different varieties grown in one place.

Comments on the Zeleny Method. The technique of the Zeleny method is quite simple, but the analytical error is appreciably higher than that of the Kjeldahl method. Unlike the latter, the Zeleny method gives higher errors for flours than for wheats. Flours are more difficult to test than wheats by the Zeleny method because it is not easy to paste them up thoroughly while the dispersing agent is being added. A comparison of the errors of the Kjeldahl and the Zeleny methods can be made from the following standard errors of duplicate determinations, which are recorded in terms of Kjeldahl protein:

	25 Varieties		Thatcher	
	Kjeldahl	Zeleny	Kjeldahl	Zeleny
Wheat	0.07	0.09	0.09	0.15
Flour	0.11	0.17	0.07	0.25

With the Zeleny method the greater part of the error appears to lie in the dispersing process, but it is reasonable to believe that further investigations may result in improvements that will materially lower the analytical error.

The cost of operating the Zeleny method is much lower than that of the Kjeldahl method, and the amount of laboratory space required is much smaller. The analytical operations of the Zeleny method can be carried out very rapidly, but more time is required for the preparation of wheat samples for this method than for the Kjeldahl procedure. The Zeleny method has special promise in field laboratories where it is not possible to install conventional protein apparatus.

Summary

The photometric method developed by Zeleny for determining protein content in wheat and flour has been examined to determine whether it gives results that are more closely related to loaf volume than are conventional Kjeldahl data. The Zeleny correlations, both within one variety (50 samples of Thatcher: wheat, − 0.88; flour, − 0.88) or between 25 varieties (wheat, − 0.75; flour, − 0.78), were not as high as the corresponding Kjeldahl correlations (0.88, 0.90; 0.79, 0.84).

The correlations between Zeleny values and Kjeldahl protein were − 0.92 and − 0.93 for wheat and flour in the intervarietal series, and − 0.98 and − 0.99 for wheat and flour in the Thatcher series. The Zeleny method can apparently be used for predicting Kjeldahl protein in sound samples of one wheat variety grown in different places, or in samples of different varieties grown at one place. It is improbable that a satisfactory general equation for all samples can be developed.

The evidence strongly suggests that the Zeleny procedure is measuring some relatively constant proportion of the total protein and that this protein fraction is concentrated largely in the endosperm.

The method is very simple, but, at the present time, the analytical error is higher than that of the Kjeldahl test.

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IMPROVEMENTS IN THE DETERMINATION OF STARCH IN CORN AND WHEAT

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Methods for the determination of starch in materials of relatively high starch content, such as cereal foods, grains, and stock feeds, have been studied by the Association of Official Agricultural Chemists for the past several years. Present official methods ("Methods of Analysis, A.O.A.C.," 5th ed., 1940) for grain and stock feeds include (1) direct acid hydrolysis, (2) diastase method with subsequent acid hydrolysis, and (3) the latter with provision for the removal of interfering polysaccharides. The first of these methods is not applicable in the presence of pentosans known to occur in grains unless a large correction involving a separate analytical determination is made for pentosans. The second method and, especially, the third method are laborious and extremely time-consuming. These methods are usually avoided in routine analyses by industrial companies because of the time and labor involved. Under "Cereal Foods," the Rask method is listed by the Association of Official Agricultural Chemists as a tentative method, applicable to uncooked cereal products. This method is empirical, requiring very careful timing and strict adherence to every detailed direction. All of the above methods of the Association of Official Agricultural Chemists are repeated in the "Cereal Laboratory Methods, A.A.C.C." (4th ed., 1941). The unsatisfactory status of these methods is recognized by the continuing work of referees in the Association of Official Agricultural Chemists; and the most recent report (A.O.A.C., 1944) recommends the Hopkins (1934) revision of the Mannich-Lenz (1920) polarimetric procedure as a tentative method for cereal foods. Etheredge (1941) has surveyed starch methods, and Hopkins (1934) presents an extensive review of the literature.

In our work the polarimetric procedure of Hopkins has been studied in some detail, and results obtained by this procedure have been compared with those obtained by the diastase and direct acid hydrolysis methods on pure starches. The polarimetric procedure was applied to corn and wheat, using the factors found in a study of pure starches, and the results on a large number of samples were compared with those by the diastase method. A pretreatment of the ground grains was developed which removed interfering materials. Satisfactory agreement

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between the polarimetric and diastase methods was obtained with corn and wheat by this means.

Experimental

Starches. The use of pure starch as a standard for evaluating analytical methods became justifiable only after a satisfactory method was developed for determining the true moisture content of this material. Water forms by far the largest nonstarch constituent, so that even a minor error in its estimation is of importance. The methods for moisture described by Sair and Fetzer (1942) were shown to be accurate within a few tenths of 1%. Other possible nonstarch constituents are fatty materials, protein, ash, and pentosans.

Table I describes the seven starches used in this work and gives

TABLE I

STARCH SAMPLES USED

No.	Type	Preparation	Moisture content
			%
1	Corn	Commercial	11.67
2	Corn	Commercial	11.08
3	Waxy corn	Laboratory Pilot Plant from Iowa 939	12.02
4	Wheat	Laboratory Pilot Plant from Dawson	12.10
5	Wheat	Laboratory from Turkey	9.88
6	Tapioca	Commercial	12.12
7	Potato	Commercial	13.43

results for moisture by the toluene distillation or vacuum oven methods of Sair and Fetzer. The results for other nonstarch constituents shown in Table II are the averages of closely agreeing duplicate or triplicate determinations. Official methods of the Association of Official Agricultural Chemists were followed where possible. Ashing was carried out at 700°C for 3 hr. The air-dried starches were extracted for 48 hr in a Soxhlet apparatus with absolute methanol. In determining the fatty acid left on acid hydrolysis, 10 g of starch were heated on the boiling water bath for 2 hr in 220 ml of 0.7*N* hydrochloric acid. The fatty acid in the residue from acid hydrolysis was extracted with petroleum ether, titrated with alkali, and calculated as oleic acid. After ashing with magnesium nitrate, phosphorus was determined colorimetrically, using stannous chloride as the reducing agent. Pentosans were determined as furfural by distillation with 12% hydrochloric acid, as in the A.O.A.C. method, but, because pure glucose yields products which give a precipitate with phloroglucinol when this procedure is used, the furfural produced was determined with thiobarbituric acid (Bailey, 1937) after a redistillation to eliminate interfering

TABLE II

NONSTARCH CONSTITUENTS (DRY BASIS)

Constituents	Sample						
	Corn	Corn	Waxy corn	Wheat	Wheat	Tapioca	Potato
	1	2	3	4	5	6	7
	%	%	%	%	%	%	%
Nitrogen	.089	.043	.033	.040	.051	.008	.012
Protein (N × 6.25)	.56	.27	.20	.25	.32	.05	.08
Ash	.10	.08	.07	.13	.14	.10	.32
Fatty acid (calculated as oleic, from titration of acid hydrolysis residue)	.68	.67	.08	.49	.51	.12	.01
Pentosans	—	.0	—	—	.0	—	.0
Phosphorus	.019	.016	.011	.048	.059	.011	.087
Methanol extract	.77	.75	.26	.39	.37	.22	.04
Fatty acid in extract (by titration)	—	.49	.09	.13	.11	.07	.02
Nitrogen in extract	.016	.007	.007	.008	.007	.001	.000
Phosphorus in extract	.005	.007	.001	.022	—	.001	.002
Fatty acid in residue from extraction	—	.04	—	—	.32	—	—
Phosphorus in residue from extraction	.015	.010	.007	.033	.048	.008	.082

substances. Unpublished work of this laboratory, as well as the work of others (Launer and Wilson, 1939), has shown that this procedure is necessary to eliminate interfering substances, such as hydroxymethyl furfural.

The differences between the methanol extract and the fatty acid found in the residue from acid hydrolysis are insignificant in calculating the sum of all nonstarch constituents. Except in the case of the wheat starches, the amount of methanol extract is always the greater, indicating that other substances besides fatty acid are extracted. The assumptions have been made in calculating the nonstarch constituents that all of the nitrogen in the starch is from protein, that all of the phosphorus is a part of the starch structure, and that all of the free fatty acid found on acid hydrolysis is a nonstarch constituent. Other assumptions of equal probability may be made, but because of the high purity of these starches, the figures for total percentage of starch would not be appreciably altered by these other assumptions.

The figures for nonstarch constituents and starch by difference are given in Table III. In Table IV are shown the starch results obtained by four methods. In making these determinations, 70% instead of 10% alcohol was used to remove sugars; a malt syrup of high diastatic power was used in place of the malt extract; the Scales method (Isbell, 1940) was used for sugar; and the factor 0.92 was used in place of 0.90

TABLE III

SPECIFIC ROTATION OF STARCH

Determination	Sample						
	Corn	Corn	Waxy corn	Wheat	Wheat	Tapioca	Potato
	1	2	3	4	5	6	7
Fatty acid by acid hydrolysis, %	.68	.67	.08	.49	.51	.12	.01
Protein (N × 6.25), %	.56	.27	.20	.25	.32	.05	.08
Ash, %	.10	.08	.07	.13	.14	.10	.32
Total nonstarch, %	1.34	1.02	.35	.87	.97	.27	.41
Starch by difference, %	98.66	98.98	99.65	99.13	99.03	99.73	99.59
Observed rotation, $CaCl_2$ soln, 1-dm tube, 2-g sample in 100 ml	4.016	4.010	4.062	4.012	3.985	4.060	4.058
Specific rotation $[\alpha]_D$,°	203.5	202.6	203.8	202.4	201.2	203.5	203.7

TABLE IV

COMPARISON OF METHODS OF DETERMINING STARCH

Method	Sample						
	Corn	Corn	Waxy corn	Wheat	Wheat	Tapioca	Potato
	1	2	3	4	5	6	7
	%	%	%	%	%	%	%
Starch by difference	98.7	99.0	99.6	99.1	99.0	99.7	99.6
Starch by polarimeter ($[\alpha]_D = 203°$)	98.9	98.8	99.8	98.8	98.2	100.0	99.9
Starch by acid hydrolysis (factor 0.92)	97.2	97.8	98.6	97.6	97.9	98.9	99.3
Starch by diastase (factor 0.92)	97.4	98.5	99.7	98.8	98.5	99.8	99.8

in converting from sugar to starch. The factor 0.92 was derived by treating pure dextrose (National Bureau of Standards' No. 41) with acid under the conditions used in both the direct acid hydrolysis and diastase methods. The use of the factor 0.93 instead of 0.90 has been suggested by Noyes (1904), Etheredge (1941), and many others. The agreement shown in Table IV between the starch by difference and the starch by diastase further supports the factor 0.92. The rotations of the starches were measured by reading the rotation of the dispersion of 2 g of starch in 100 ml of the calcium chloride solution described later, using a 1-dm tube. The specific rotations, $[\alpha]_D$, of the starches were calculated from the starch contents as determined by difference and the observed rotations. The average specific rotation of 203° was used in all further work, since the differences shown by the various kinds of starch do not exceed the possible experimental error. The

specific rotation calculated for the two wheat starches, is slightly lower than 203°, but until a larger number of starches are studied this average value is recommended. This value of 203° is slightly higher than the commonly used value of 200° (Mannich-Lenz, 1920).

The agreement between the figures for starch determined by diastase and starch determined by difference was satisfactory, whereas the figure for starch by acid hydrolysis was consistently lower. The average values for the seven starches were: by difference, 99.25%;[2] by diastase, 98.93%; and by acid hydrolysis, 98.17%. No effort was made to depart from the official methods for the acid hydrolysis and diastase methods in order to obtain higher values.

Corn and Wheat. Determinations of the starch content of corn and wheat were carried out using essentially the A.O.A.C. tentative polarimetric method and the A.O.A.C. diastase method with the modifications previously described. Experiments on the fineness of grinding required to obtain a complete dispersion of the starch of these grains in calcium chloride solution showed that grinding through the ½-mm screen of a Wiley mill was satisfactory. Comparable and equally satisfactory results were obtained by grinding corn on a Bauer Brothers mill (3,600 rpm attrition mill) and by grinding wheat on a Labconco mill.

Comparison of the results by the polarimetric and diastase methods on a series of 30 corn samples showed fairly satisfactory agreement. The average by the polarimetric method was 71.0% starch, and that by the diastase method (factor 0.92) was 69.9%. Although the polarimetric procedure, as described in the Journal of the Association of Official Agricultural Chemists, is much easier and faster than the diastase procedure, the modification described by Clendenning[3] for use on wheat flour offers a further advantage in speed. A comparison made between these two polarimetric procedures showed on 23 samples of corn an average of 69.5% starch by the A.O.A.C. procedure (using an alcohol wash) and 69.3% starch by the Clendenning procedure (in which 5.0 ml of 2.5% stannic chloride is added to precipitate proteins in place of washing). It is apparent that any sugar present in the calcium chloride dispersion does not appreciably affect the results obtained for starch.

When the diastase and polarimetric methods of the Association of Official Agricultural Chemists were used on wheat, the diastase method gave average results for starch that were 2.5% higher than those obtained by the polarimetric procedure. Differences in results by the two methods were especially marked when fractions from wheat milling,

[2] All results are calculated to the moisture-free basis.
[3] Dr. K. A. Clendenning very kindly furnished directions for his method of determining starch in wheat flour (unpublished).

such as bran and shorts, were analyzed. It was found that the use of stannic chloride or other protein precipitant was of no assistance on many samples since the solutions could not be filtered easily and were turbid after filtration. Balch and Phillips (1941), in a study of the determination of starch in sweet potatoes, found that an alkaline wash removed interfering materials. This procedure was found to work very well with wheat, and the alkaline wash was shown experimentally not to change the rotation of pure starch. The ether and alcohol washes recommended by the Association of Official Agricultural Chemists were replaced by three washes with 0.05*N* sodium hydroxide, followed by one wash with 0.5*N* acetic acid and two washes with alcohol. Using this alkaline wash in the polarimetric procedure, analyses of 43 samples of wheat grown in 1940 and 1942 gave 63.9% for the average starch content in comparison with 65.4% by the diastase procedure; this was a marked improvement over previous results.

The effect of the alkaline wash on the diastase determination was also investigated. For a group of 46 wheats grown in 1941, the average starch content was 65.1%, which was exactly the same as the average starch content by the polarimetric procedure using an alkaline wash. In making these comparisons, the average difference between duplicates on 42 samples was 0.27% for both methods, and the standard error of a single determination was 0.19% for the polarimetric and 0.27% for the diastase. The maximum difference between methods was 1.8%. On the other four samples, duplicates by the diastase method differed by more than 1% and were not included in the average since additional determinations were necessary. Three other samples, in which the ratio of starch to nitrogen differed from that expected, were rerun by the polarimetric method. On two of these samples in the repeated determinations the starch value was increased, making the ratio of starch to nitrogen more nearly that which was expected.

Improved Polarimetric Method

The details of the final methods used for determining starch in corn and wheat are as follows:

Reagents

Calcium chloride solution: Dissolve 546 g of $CaCl_2 \cdot 2H_2O$ in distilled water and dilute to one liter. Adjust density to 1.30 at 20°C. Add glacial acetic acid to bring to pH of 2.5 ± 0.3.

Stannic chloride solution: Dissolve 2.5 g of $SnCl_4 \cdot 5H_2O$ in 97.5 g of the $CaCl_2$ solution.

Sodium hydroxide solution: 0.05*N*.

Acetic acid solution: 0.5*N*.

Ethanol: 70% by volume.

Determination

Corn. Grind the sample through a 0.5-mm Wiley screen, or to an equivalent fineness, and weigh 2 g into a 250-ml beaker. Add 10 ml H_2O, and stir to wet the

sample. Add 70 ml of $CaCl_2$ solution. Cover with watch glass, and bring to a boil in 5 min on a hot plate. Boil 15 min, stirring as needed to keep the sample from sticking to the side of the beaker. The stirring will also help to prevent bumping and foaming, but it may be necessary to blow into the beaker at times to prevent foaming. Cool quickly, and transfer to a 100-ml volumetric flask with $CaCl_2$ solution. Add 5 ml of $SnCl_4$ solution and fill to the mark with $CaCl_2$ solution. Mix well, and filter on a folded No. 2 Whatman filter paper, discarding the first 15 ml of the filtrate. Collect an additional portion of the filtrate in a separate receiver and polarize in a 10-cm tube.

Calculate the starch content as follows:

$$\text{Percentage of starch} = \frac{A \times 100 \times 100}{203 \times S}$$

where A = observed rotation,
S = sample weight.

Wheat. Grind the sample through the Wiley 0.5-mm screen and weigh 2 g into a round-bottomed, 50-ml, heavy-duty, centrifuge tube. Add 10 ml of 0.05*N* NaOH and stir thoroughly. Centrifuge, decant, and wash twice more with alkali. Similarly, wash once with 0.5*N* acetic acid and twice with 70% alcohol. Transfer to a 250-ml beaker with 10 ml of water; add 70 ml $CaCl_2$ solution; and disperse as for corn. Cool quickly; transfer to a 100-ml volumetric flask; and fill to the mark with $CaCl_2$ solution. Mix well, and filter on a folded No. 4 Whatman filter paper (12.5 cm), discarding the first 15 ml of the filtrate. Complete as for corn.

Discussion

The speed with which determinations may be completed is one of the greatest advantages of the polarimetric method. Samples may be ground, weighed, washed, dispersed, and completely analyzed within 1¾ hr. A total of eight hours is required to complete a determination by the official diastase method. Over a period of days, one analyst can complete in an 8-hr working day, 14 polarimetric determinations or 10 diastase determinations on wheat using the alkaline wash, and 24 or 16, respectively, on corn using the stannic chloride precipitation in the polarimetric and the alcohol wash in the diastase method.

The differences between the proteins, starches, and pentosans of corn and wheat are responsible for the different pretreatments used for these two grains. The corn could not be washed with alkali without a loss of starch in the wash waters, as shown by an iodine test. With wheat, no loss of starch has been observed. After the addition of stannic chloride to wheat, the solution could not be filtered satisfactorily, and the filtrate was frequently too cloudy to be measured in a polarimeter. No attempt has been made to show how these differences are related to the known properties of corn and wheat proteins.

Closer agreement between the starch by difference and the starch by diastase could be obtained in the case of the seven pure starches if the factor of 0.93 were used to convert sugar to starch. This factor has been suggested by others, but, as stated previously, several experiments on pure dextrose showed that approximately 98% recovery was obtained when this substance was put through the acid hydrolysis used in the starch determination. The lower values found for the pure

starches by the direct acid hydrolysis and the diastase methods may indicate difficulty in obtaining the complete hydrolysis of starch to reducing sugars.

The good agreement obtained between the two methods—one physical and one chemical—lends support to both. The polarimetric method is empirical, since the specific rotation of starch is obtained by measurements on noncrystalline materials, and is to some extent dependent on the accuracy of analysis for the nonstarch constituents. Use of this method on many samples of corn and wheat has shown it to be satisfactory for calculating yields of starch or products from starch. Results obtained by this method are reproducible within about 1%. The accuracy of the method has not been determined, but it is believed that it gives results within 1% of the true values.

The polarimetric method may be applicable to many other grains and in this laboratory it has been used on sorghums, oats, barley, and rye. Since no study was made on pure starches from these grains, it was necessary to use the specific rotation of 203° found for corn and wheat. In applying the polarimetric method to other grains, special treatments, similar to the alcohol and other washes, the stannic chloride addition, or the alkaline wash, may be found necessary, but no data have been obtained on these applications to date.

Summary

Seven samples of starch, comprising two from corn, two from wheat, and one each from waxy corn, tapioca, and potato, were analyzed for nonstarch constituents.

The specific rotation, $[\alpha]_D$, of all these starches was found to be 203° in calcium chloride dispersion, when corrected for the nonstarchy material.

Modifications of the polarimetric method for starch in corn and wheat were studied and procedures devised which, for these two grains, gave results agreeing closely with those obtained by the diastase-acid hydrolysis method. These improved polarimetric procedures are described.

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A NOTE ON THE COMPARATIVE EFFICIENCY OF L-ASCORBIC ACID AND POTASSIUM BROMATE AS DOUGH CONDITIONERS

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The oxidizing effect of l-ascorbic acid in bread doughs has been the subject of several investigations. Jørgensen (1935, 1939) and Feaster and Cathcart (1941) showed that l-ascorbic acid was equivalent to potassium bromate as a dough conditioner on a weight-for-weight basis. Recent experiments in this laboratory have shown that l-ascorbic acid is not quite as effective as potassium bromate as a dough conditioner.

Baking tests were conducted, using the procedure outlined in Cereal Laboratory Methods (4th ed., 1941). Potassium bromate and l-ascorbic acid were compared in these baking tests. Three regular commercial-run flours were used in the tests: (1) short patent flour; (2) stuffed straight flour; and (3) whole wheat flour. All of these flours were treated with Agene at the mill. The results given in Table I show that l-ascorbic acid affects the volume and improves the grain, texture, and crumb color of the loaves in a manner similar to bromate. However, all of the data show that it is necessary to use approximately one and one-half times as much l-ascorbic acid as bromate to obtain the

TABLE I

COMPARISON OF L-ASCORBIC ACID AND POTASSIUM BROMATE AS DOUGH CONDITIONERS
(All flours treated with Agene)
Quantities given in milligrams per 100 grams of flour

	Loaf volume	Grain	Texture	Crumb color
Short patent flour				
Control	680	Slightly elongated; slightly attenuated	Soft	Very slightly creamy
1.0 mg $KBrO_3$	660	Improved attenuation	Soft	Creamy white
1.0 mg l-ascorbic acid	680	Slightly improved attenuation	Soft	Shade darker than bromate loaf
1.5 mg l-ascorbic acid	665	Improved attenuation	Soft plus	Shade brighter than bromate loaf
2.0 mg l-ascorbic acid	675	Improved attenuation	Soft plus	Shade brighter than bromate loaf
Stuffed straight flour				
Control	655	Coarse, poor attenuation	Soft minus	Creamy and dull
1.0 mg $KBrO_3$	705	Slightly elongated; much improved attenuation	Soft plus	Much brighter than control
1.0 mg l-ascorbic acid	665	Coarse; slightly improved attenuation	Soft	Shade brighter than control
1.5 mg l-ascorbic acid	695	Slightly elongated; improved attenuation	Soft plus	Much brighter than control
2.0 mg l-ascorbic acid	680	Slightly elongated; improved attenuation	Soft plus	Much brighter than control
Whole wheat flour				
Control	560	Coarse	Harsh	Satisfactory
2.0 mg $KBrO_3$	625	Improved attenuation	*	Much brighter than control
2.0 mg l-ascorbic acid	590	Slightly improved attenuation	**	Shade brighter than control
3.0 mg l-ascorbic acid	620	Improved attenuation	*	Much brighter than control
4.0 mg l-ascorbic acid	590	Improved attenuation	*	Much brighter than control

* Much improved over control.
** Slightly improved over control.

same results. This was verified by other experiments in the present study. During the course of the experiments, d-iso-ascorbic acid was found to be ineffective as a dough conditioner, confirming earlier work by Feaster and Cathcart (1941).

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BOOK REVIEWS

The Chemistry and Technology of Food and Food Products. Volume I. Prepared by a group of specialists under the editorship of Morris B. Jacobs. 952 pp. Interscience Publishers, Inc., New York, N. Y. 1944. Price $10.50.

This book is the first of two volumes designed to survey the fields of food chemistry and technology. It includes the contributions of 21 collaborators selected for their qualifications to present an authoritative discussion of the many aspects of this subject. Divided into two parts, "Fundamentals" and "Foods," this first volume is devoted mainly to the chemistry of foods and will be followed shortly by the second volume in which the technological aspects will be emphasized.

Ten subjects are considered in the section dealing with "Fundamentals." These are: (1) The Physical Chemistry of Foods (St. John), (2) The Carbohydrates (Degering), (3) Lipids (Baizer and Zahnd), (4) Some Aspects of the Chemistry of Amino Acids in Proteins (Zahnd and Baizer), (5) Enzymes (Stern), (6) Vitamins, Vitagens, and Hormones (Rosenberg), (7) Mineral Matters and Other Inorganic Food Adjuncts (Carr), (8) Coloring Matters in Foods (Jablonski), (9) The Digestion and Fate of Foodstuffs (Wilhelmi), and (10) Food Spoilage and Food Poisoning (Halvorson).

This is an imposing array of titles and indicates the intent of the editor to present a truly comprehensive treatise. In some instances, however, the allotted space is inadequate for the subject material, and important omissions will be noted. The discussion of physical chemistry is essentially a series of definitions, while the chapter dealing with carbohydrates will be of limited value to the reader seeking information about dextrins and starches. The amino acids and proteins are discussed more extensively and include references to recent literature of considerable importance to the chemistry of foods. Lipids are rather sketchily considered, and the subject of enzymes pays little attention to practical applications. Rancidity is barely mentioned, and it is hoped that Volume II will include a discussion of this important phenomenon. Many food technologists will want to supplement these chapters with other sources of information. Vitamins are reviewed almost entirely from the purely chemical standpoint, with no reference to the phases of methodology so important to many workers in food fields. This chapter is essentially a condensation of the book recently written by the same author. The chapters dealing with minerals, food colors, digestive processes, and food spoilage are of general interest. While brief, they cover the salient points prerequisite to an appreciation of food problems.

Part II, entitled "Foods," presents discussions of 12 food fields. Here the reader will find specific information relating to: (1) Milk, Cream, and Dairy Products (Jacobs), (2) Meat and Meat Products (Urbain), (3) Fish, Shell Fish, Crustacea (Stansby), (4) Poultry and Eggs (Pennington), (5) Edible Oils and Fats (Bailey), (6) Cereal Grains (Geddes), (7) Baking and Bakery Products (Cathcart), (8) Vegetables, Mushrooms, Nuts, and Fruits (Lee), (9) Carbohydrate and Sugar Foods (Degering), (10) Confectionery and Cacao Products (Schoen), (11) Coffee and Tea (Ukers), and (12) Flavors, Spices, and Condiments (Worrell).

The subject of dairy products describes some of the physical and physiochemical properties of milk and 17 types of milk products. In many instances, the treatment is rather brief. Numerous tables carry information about the composition of dairy products. The subject of nutrition is rather incomplete, less than half a page being devoted to the vitamins. Considerable information has been obtained since 1938, the most recent source of information presented. Of greatest interest to the cereal chemist will be the chapters dealing with cereal grains and bakery products. These two chapters comprise nearly one-sixth of the volume, reflecting the extensive treatment given these items. The chapter on cereal grains includes considerations of production, structure, classification, and chemical composition. Barley, buckwheat,

corn, oats, rice, rye, and wheat are separately treated. Brief consideration is also given to cottonseed, peanut, and soybean flours. The chapter on baking and bakery products describes the ingredients and reactions taking place in the production of bread, cakes, cookies, etc. Such subjects as staleness, nutritive properties, and microbiology of bakery products are included. The chapter dealing with carbohydrate and sugar foods is out of place in the general plan of the book. It is an extension of the chapter by the same author appearing under "Fundamentals" and the reason for its inclusion under "Foods" is rather obscure. Those seeking knowledge about the practical aspects of carbohydrate foods will be disappointed.

An evaluation of the first volume with respect to its utility is made somewhat difficult by the type of arrangements planned for the two-volume set. In many instances, a discussion of the chemistry and technology of foods should go hand in hand, and the differentiation placing one phase in one volume and the second in another has certain disadvantages. The appearance of brevity in many of the chapters may be removed by the treatment in the second volume. This does not remove, however, the rather disconcerting situation as illustrated in the chapter on Confectionery and Cacao Products, where definitions for cacao, cocoa, and chocolate are presented, together with the statement that standards for different cacao products are discussed in Volume II, Chapter 18. From the convenience standpoint, it would have seemed preferable to discuss one subject completely, rather than attempt a rather indefinite segregation in two volumes.

Despite many of the limitations, the editor and collaborators are to be congratulated on their attempt to consolidate the many aspects of food chemistry and technology. Many of the chapters are well referenced, and any information not found in the descriptive parts can be sought conveniently by reference to the selected bibliography. The present volume, together with the one which is expected to appear shortly, should occupy a prominent place in the library of all interested in foods.

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Chemistry and Industry of Starch. Edited by Ralph W. Kerr. 472 pp. Academic Press, Inc., New York, N. Y. 1944. Price $8.50.

The editor of this book, in which the accumulated knowledge of starch and products derived therefrom is brought up to date, is assisted by 14 co-authors, each a specialist in some phase of starch chemistry. Greater emphasis is given the American viewpoint and practice of technology than was done in most earlier books in this field. The text is not intended to be comprehensive, since a single volume scarcely offers sufficient space to do this with the many divisions of the subject. The editor expresses the hope that this survey "will be of benefit not only to the carbohydrate chemist but to the technologist, whose daily task is concerned with more efficient production of starch products and with their more efficient utilization."

The subject matter is contained in five sections comprising 22 chapters. The section outline which follows shows the order of treatment of the various topics and indicates the scope of the text.

Section I. Occurrence in nature—source of starch; size and shape of starch granules; the hilum; striations; swelling of granules in water; description of individual starches; photomicrographs of various starches.

Section II. Preparation—the manufacture of corn, modified corn, tapioca, wheat, white potato, sweet potato, arrowroot and sago starches; evaluation of modified starches in practice.

Section III. Properties—physical and chemical properties of starch (composition and structure).

Section IV. Reactions—the hydrogen bond in starch as a basis for interpreting its behavior and reactivity; derivatives of starch; oxidation of starch; dextrinization; manufacture of dextrins; acid hydrolysis of starch; the amylases (properties and production); modification of starch by enzymes; miscellaneous reactions (iodine, formaldehyde, other aldehydes, hydrogenation).

Section V. Uses—uses of starch in paper manufacture; uses of starch and starch products in the fermentation industries; use of starch and starch products in foods; use of starch products in the textile industry; starch adhesives.

The text maintains a good balance between fundamental and applied knowledge and should inspire more fundamental research leading to new industrial applications of starch. Author and subject indices are included.

The editor, authors, and publishers are to be congratulated in providing this timely text on starch which will be a valuable reference work for everyone interested in carbohydrate chemistry.

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Micromeritics. J. M. DallaValle. 428 pp. Pitman Publishing Company, New York, N. Y. 1943. Price $8.50.

The author has coined the word "micromeritics" to designate the technology of fine particles, the term "fine" being defined to cover the range of particle diameters from 10^{-1} to 10^{5} μ (10^{-4} to 100 mm). To quote the author, "This text is intended as a guide to the general subject of the behavior and characteristics of small particles. It brings together a mass of widely scattered information on methods of particle-measurement, size-distributions, packing arrangements, and a general theory concerning the physical properties of finely divided substances." For this reason alone, it will probably be a welcome addition to the technologist's reference library.

The subjects covered range from a rigorous mathematical treatment of the dynamics of small particles through discussions on their electrical, optical, sonic, chemical, and thermodynamic properties to a dissertation on muds and slurries. Because of the broad nature of the subject covered, the book assumes somewhat of an incoherent nature and, for this reason, will find its greatest acceptance as a reference rather than as a textbook.

It is felt that many of the mathematical presentations are "thrown" at the reader without proper introduction and explanation; for instance, the equations of motion of a particle given on page 19 are simply stated to be the equations of motion in two dimensions. Not until the reader has inspected the equations does he learn that they are those for the special case in which one of the dimensions is the vertical, and the particle is subject to the force due to gravity. Only after further inspection is it revealed that the motion is in a medium in which the density is different from zero. Perhaps this criticism should be overlooked in view of the volume of material covered, but improper presentations of this kind become confusing.

Because of the author's election to treat the subject in a rigorous manner, the student who has had no more than an "exposure" to higher mathematics will probably find himself beyond his depth on numerous occasions.

The 38-page bibliography at the end of the book will be well accepted.

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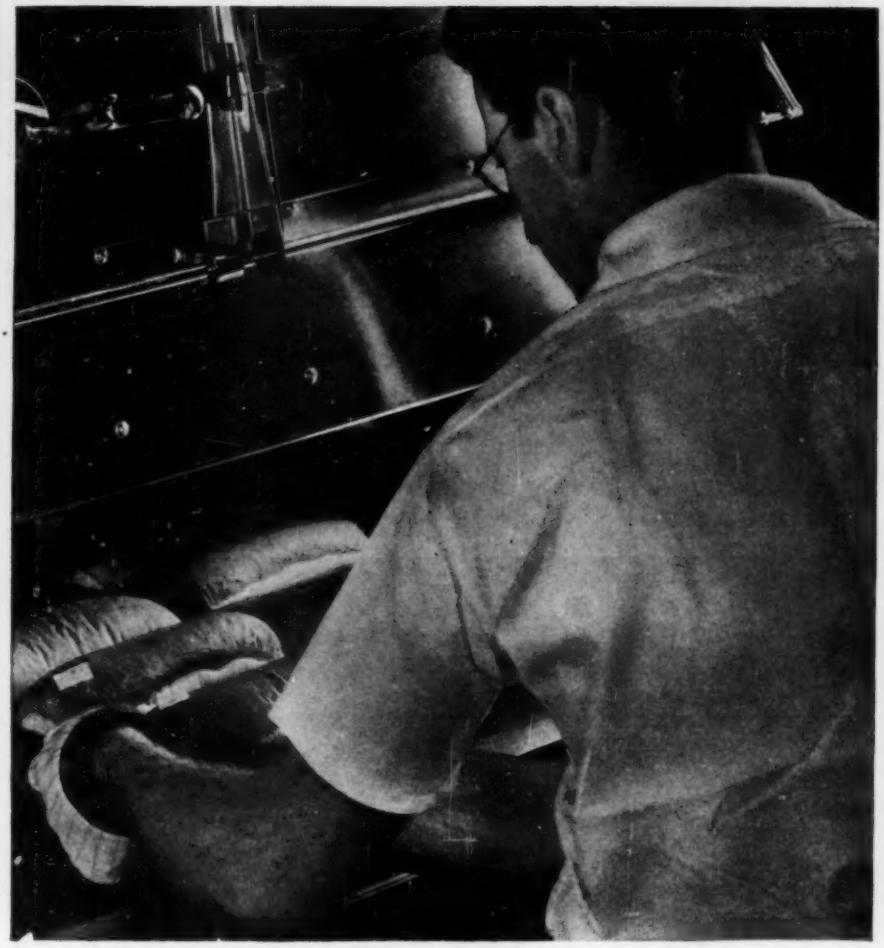

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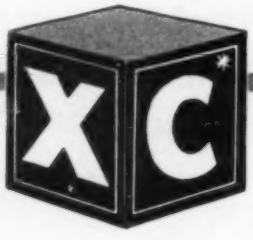

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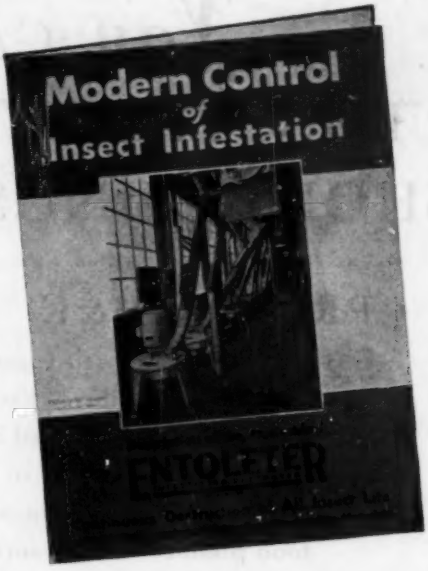

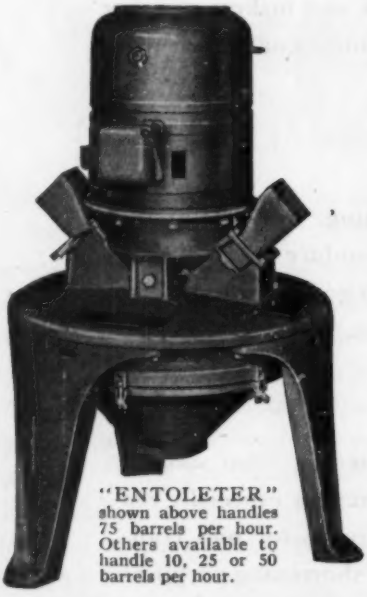

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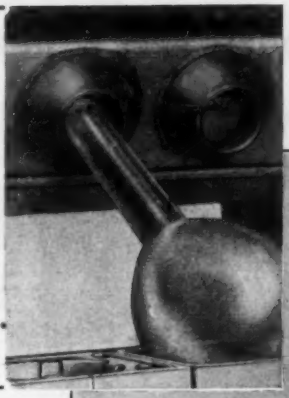

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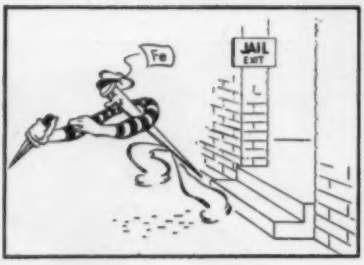

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